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 * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 15:09:55 ON 09 MAY 2003

=> fil .bec
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 15:10:24 ON 09 MAY 2003
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s ikk?
FILE 'MEDLINE'
L1         1687 IKK?

FILE 'SCISEARCH'
L2          818 IKK?

FILE 'LIFESCI'
L3          356 IKK?

FILE 'BIOTECHDS'
L4           34 IKK?

FILE 'BIOSIS'
L5          755 IKK?

FILE 'EMBASE'
L6          533 IKK?

FILE 'HCAPLUS'
L7          782 IKK?

FILE 'NTIS'
L8           36 IKK?

FILE 'ESBIOBASE'
L9          478 IKK?

FILE 'BIOTECHNO'
L10         366 IKK?

FILE 'WPIDS'
L11          66 IKK?

TOTAL FOR ALL FILES
L12        5911 IKK?

=> s l12(5a)bind?
FILE 'MEDLINE'
        657442 BIND?
L13          54 L1 (5A)BIND?

FILE 'SCISEARCH'
        610471 BIND?
L14          58 L2 (5A)BIND?

FILE 'LIFESCI'
        224237 BIND?
L15          32 L3 (5A)BIND?
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FILE 'BIOTECHDS'
         32039 BIND?
L16          7 L4 (5A)BIND?

FILE 'BIOSIS'
        611764 BIND?
L17         69 L5 (5A)BIND?

FILE 'EMBASE'
        569112 BIND?
L18         39 L6 (5A)BIND?

FILE 'HCAPLUS'
        977029 BIND?
L19         67 L7 (5A)BIND?

FILE 'NTIS'
         14498 BIND?
L20          0 L8 (5A)BIND?

FILE 'ESBIOBASE'
        210096 BIND?
L21         39 L9 (5A)BIND?

FILE 'BIOTECHNO'
        273603 BIND?
L22         31 L10(5A)BIND?

FILE 'WPIDS'
        240372 BIND?
L23         14 L11(5A)BIND?

TOTAL FOR ALL FILES
L24        410 L12(5A) BIND?

=> s spa-1
FILE 'MEDLINE'
          3246 SPA
       2992666 1
L25         35 SPA-1
                (SPA(W)1)

FILE 'SCISEARCH'
          2399 SPA
       3032005 1
L26         29 SPA-1
                (SPA(W)1)

FILE 'LIFESCI'
           703 "SPA"
        511646 "1"
L27         15 SPA-1
                ("SPA"(W)"1")

FILE 'BIOTECHDS'
           139 SPA
        153466 1
L28          4 SPA-1
                (SPA(W)1)

FILE 'BIOSIS'
          2628 SPA
       2889055 1
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L29          37 SPA-1
                  (SPA(W)1)

FILE 'EMBASE'
          3318 "SPA"
       1827106 "1"
L30          29 SPA-1
                  ("SPA"(W)"1")

FILE 'HCAPLUS'
          2684 SPA
       7532039 1
L31          41 SPA-1
                  (SPA(W)1)

FILE 'NTIS'
           225 SPA
        500674 1
L32           4 SPA-1
                  (SPA(W)1)

FILE 'ESBIOBASE'
           781 SPA
        825085 1
L33          19 SPA-1
                  (SPA(W)1)

FILE 'BIOTECHNO'
           745 SPA
        626644 1
L34          15 SPA-1
                  (SPA(W)1)

FILE 'WPIDS'
          1123 SPA
       6681503 1
L35           7 SPA-1
                  (SPA(W)1)

TOTAL FOR ALL FILES
L36        235 SPA-1

=> s l36 and bind?
FILE 'MEDLINE'
        657442 BIND?
L37          17 L25 AND BIND?

FILE 'SCISEARCH'
        610471 BIND?
L38          11 L26 AND BIND?

FILE 'LIFESCI'
        224237 BIND?
L39           4 L27 AND BIND?

FILE 'BIOTECHDS'
         32039 BIND?
L40           2 L28 AND BIND?

FILE 'BIOSIS'
        611764 BIND?
L41           6 L29 AND BIND?

FILE 'EMBASE'
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        569112 BIND?
L42           9 L30 AND BIND?

FILE 'HCAPLUS'
        977029 BIND?
L43          19 L31 AND BIND?

FILE 'NTIS'
         14498 BIND?
L44           0 L32 AND BIND?

FILE 'ESBIOBASE'
        210096 BIND?
L45           2 L33 AND BIND?

FILE 'BIOTECHNO'
        273603 BIND?
L46           7 L34 AND BIND?

FILE 'WPIDS'
        240372 BIND?
L47           1 L35 AND BIND?

TOTAL FOR ALL FILES
L48          78 L36 AND BIND?

=> s l12 and l36
FILE 'MEDLINE'
L49           0 L1 AND L25

FILE 'SCISEARCH'
L50           0 L2 AND L26

FILE 'LIFESCI'
L51           0 L3 AND L27

FILE 'BIOTECHDS'
L52           1 L4 AND L28

FILE 'BIOSIS'
L53           0 L5 AND L29

FILE 'EMBASE'
L54           0 L6 AND L30

FILE 'HCAPLUS'
L55           0 L7 AND L31

FILE 'NTIS'
L56           0 L8 AND L32

FILE 'ESBIOBASE'
L57           0 L9 AND L33

FILE 'BIOTECHNO'
L58           0 L10 AND L34

FILE 'WPIDS'
L59           1 L11 AND L35

TOTAL FOR ALL FILES
L60           2 L12 AND L36

=> s (l24 or l48 or l60)
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FILE 'MEDLINE'
L61          71 (L13 OR L37 OR L49)

FILE 'SCISEARCH'
L62          69 (L14 OR L38 OR L50)

FILE 'LIFESCI'
L63          36 (L15 OR L39 OR L51)

FILE 'BIOTECHDS'
L64           9 (L16 OR L40 OR L52)

FILE 'BIOSIS'
L65          75 (L17 OR L41 OR L53)

FILE 'EMBASE'
L66          48 (L18 OR L42 OR L54)

FILE 'HCAPLUS'
L67          86 (L19 OR L43 OR L55)

FILE 'NTIS'
L68           0 (L20 OR L44 OR L56)

FILE 'ESBIOBASE'
L69          41 (L21 OR L45 OR L57)

FILE 'BIOTECHNO'
L70          38 (L22 OR L46 OR L58)

FILE 'WPIDS'
L71          15 (L23 OR L47 OR L59)

TOTAL FOR ALL FILES
L72         488 (L24 OR L48 OR L60)

=> dup rem l72
PROCESSING COMPLETED FOR L72
L73          136 DUP REM L72 (352 DUPLICATES REMOVED)

=> d tot

L73  ANSWER 1 OF 136      MEDLINE                          DUPLICATE 1
TI   AF-6 controls integrin-mediated cell adhesion by regulating Rap1
     activation through the specific recruitment of Rap1GTP and SPA-
     1.
SO   JOURNAL OF BIOLOGICAL CHEMISTRY, (2003 Apr 25) 278 (17) 15232-8.
     Journal code: 2985121R. ISSN: 0021-9258.
AU   Su Li; Hattori Masakazu; Moriyama Masaki; Murata Norihito; Harazaki
     Masashi; Kaibuchi Kozo; Minato Nagahiro
AN   2003198289      IN-PROCESS

L73  ANSWER 2 OF 136      MEDLINE                          DUPLICATE 2
TI   Protection of Islets by in Situ Peptide-mediated Transduction of the
     Ikappa B Kinase Inhibitor Nemo-binding Domain Peptide.
SO   JOURNAL OF BIOLOGICAL CHEMISTRY, (2003 Mar 14) 278 (11) 9862-8.
     Journal code: 2985121R. ISSN: 0021-9258.
AU   Rehman Khaja K; Bertera Suzanne; Bottino Rita; Balamurugan A N; Mai
     Jeffrey C; Mi Zhibao; Trucco Massimo; Robbins Paul D
AN   2003113510      IN-PROCESS

L73  ANSWER 3 OF 136      MEDLINE                          DUPLICATE 3
TI   An affibody in complex with a target protein: structure and coupled
     folding.
```

SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2003 Mar 18) 100 (6) 3185-90.
Journal code: 7505876. ISSN: 0027-8424.
AU Wahlberg Elisabet; Lendel Christofer; Helgstrand Magnus; Allard Peter; Dincbas-Renqvist Vildan; Hedqvist Anders; Berglund Helena; Nygren Per-Ake; Hard Torleif
AN 2003130524 MEDLINE

L73 ANSWER 4 OF 136 MEDLINE DUPLICATE 4
TI Human T-lymphotropic virus type I tax activates I-kappa B kinase by inhibiting I-kappa B kinase-associated serine/threonine protein phosphatase 2A.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2003 Jan 17) 278 (3) 1487-93.
Journal code: 2985121R. ISSN: 0021-9258.
AU Fu De-Xue; Kuo Yu-Liang; Liu Bao-Ying; Jeang Kuan-Teh; Giam Chou-Zen
AN 2003032305 MEDLINE

L73 ANSWER 5 OF 136 MEDLINE DUPLICATE 5
TI BMS-345541 is a highly selective inhibitor of I kappa B kinase that binds at an allosteric site of the enzyme and blocks NF-kappa B-dependent transcription in mice.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2003 Jan 17) 278 (3) 1450-6.
Journal code: 2985121R. ISSN: 0021-9258.
AU Burke James R; Pattoli Mark A; Gregor Kurt R; Brassil Patrick J; MacMaster John F; McIntyre Kim W; Yang Xiaoxia; Iotzova Violetta S; Clarke Wendy; Strnad Joann; Qiu Yuping; Zusi F Christopher
AN 2003032299 MEDLINE

L73 ANSWER 6 OF 136 MEDLINE DUPLICATE 6
TI Inhibition of IkappaB kinase by a new class of retinoid-related anticancer agents that induce apoptosis.
SO MOLECULAR AND CELLULAR BIOLOGY, (2003 Feb) 23 (3) 1061-74.
Journal code: 8109087. ISSN: 0270-7306.
AU Bayon Yolanda; Ortiz Maria A; Lopez-Hernandez Francisco J; Gao Feng; Karin Michael; Pfahl Magnus; Piedrafita F Javier
AN 2003022973 MEDLINE

L73 ANSWER 7 OF 136 MEDLINE DUPLICATE 7
TI Curcumin (diferuloylmethane) down-regulates the constitutive activation of nuclear factor-kappa B and IkappaBalpha kinase in human multiple myeloma cells, leading to suppression of proliferation and induction of apoptosis.
SO BLOOD, (2003 Feb 1) 101 (3) 1053-62.
Journal code: 7603509. ISSN: 0006-4971.
AU Bharti Alok C; Donato Nicholas; Singh Sujay; Aggarwal Bharat B
AN 2003022721 MEDLINE

L73 ANSWER 8 OF 136 MEDLINE DUPLICATE 8
TI IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway.
SO Nat Immunol, (2003 May) 4 (5) 491-6.
Journal code: 100941354. ISSN: 1529-2908.
AU Fitzgerald Katherine A; McWhirter Sarah M; Faia Kerrie L; Rowe Daniel C; Latz Eicke; Golenbock Douglas T; Coyle Anthony J; Liao Sha-Mei; Maniatis Tom
AN 2003199991 IN-PROCESS

L73 ANSWER 9 OF 136 MEDLINE DUPLICATE 9
TI betaTrCP-mediated proteolysis of NF-kappaB1 p105 requires phosphorylation of p105 serines 927 and 932.
SO MOLECULAR AND CELLULAR BIOLOGY, (2003 Jan) 23 (1) 402-13.
Journal code: 8109087. ISSN: 0270-7306.
AU Lang Valerie; Janzen Julia; Fischer Gregory Zvi; Soneji Yasmina; Beinke Soren; Salmeron Andres; Allen Hamish; Hay Ronald T; Ben-Neriah Yinon; Ley Steven C

AN 2002738283 MEDLINE

L73 ANSWER 10 OF 136 MEDLINE DUPLICATE 10
TI Mixed lineage kinase LZK and antioxidant protein-1 activate NF-kappaB synergistically.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (2003 Jan) 270 (1) 76-83.
Journal code: 0107600. ISSN: 0014-2956.
AU Masaki Megumi; Ikeda Atsushi; Shiraki Eriko; Oka Shogo; Kawasaki Toshisuke
AN 2002730479 MEDLINE

L73 ANSWER 11 OF 136 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Polynucleotide encoding an I kappa B kinase binding protein Y2H14 and the recombinant protein encoded for elucidating and controlling pathways leading to inflammation and apoptosis;
using plasmid pGTB9c and plasmid pBNN132 together with an antibody
AU MARCU K B
AN 2002-17097 BIOTECHDS
PI US 6365722 2 Apr 2002

L73 ANSWER 12 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 12
TI IKK-alpha proteins nucleic acids and methods.
SO Official Gazette of the United States Patent and Trademark Office Patents, (Nov. 12 2002) Vol. 1264, No. 2, pp. No Pagination.
http://www.uspto.gov/web/menu/patdata.html. e-file.
ISSN: 0098-1133.
AU Rothe, Mike; Cao, Zhaodan; Regnier, Catherine
AN 2003:42586 BIOSIS

L73 ANSWER 13 OF 136 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 13
TI Protein-protein interactions of IkB kinases and diagnosis and treatment of inflammation and cancer and other diseases
SO PCT Int. Appl., 61 pp.
CODEN: PIXXD2
IN Cimbora, Daniel M.; Heichman, Karen; Bartel, Paul L.
AN 2002:637790 HCAPLUS
DN 137:181914

PATENT NO	KIND	DATE	APPLICATION NO.	DATE
WO 2002064736	A2	20020822	WO 2002-US194	20020104
WO 2002064736	A3	20030227		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 2003064408	A1	20030403	US 2002-35343	20020104

L73 ANSWER 14 OF 136 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New isolated protein complex comprising a first and second protein, useful for the diagnosis and treatment of disorders involved in the protein-protein interaction, such as rheumatoid arthritis, diabetes, asthma, or cancer;
recombinant protein production and sense and antisense sequence use in disease therapy and gene therapy
AU CIMBORA D M; HEICHMAN K; BARTEL P L
AN 2003-02219 BIOTECHDS
PI WO 2002064736 22 Aug 2002

L73 ANSWER 15 OF 136 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

TI Novel antiinflammatory peptide compounds comprising NEMO binding domain, useful for modulating NF-kappaB induction in a cell and for treating NF-kappaB-mediated inflammation disorders e.g., asthma, psoriasis, vasculitis;
vector plasmid expression in COS cell and transgenic animal for disease therapy
AU MAY M J; GHOSH S
AN 2003-10052 BIOTECHDS
PI US 2002156000 24 Oct 2002

L73 ANSWER 16 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Method for identifying compounds for treatment of insulin resistance.
SO Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 22, 2002) Vol. 1263, No. 4, pp. No Pagination. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133.
AU Shoelson, Steven
AN 2002:622050 BIOSIS

L73 ANSWER 17 OF 136 HCAPLUS COPYRIGHT 2003 ACS
TI Human E6-targeted protein 1 for diagnosis and treatment of cancer
SO U.S., 73 pp.
CODEN: USXXAM
IN Band, Vimla; Gao, Qingshen
AN 2002:655002 HCAPLUS
DN 137:197335

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6440696	B1	20020827	US 1999-362336	19990728
US 2003064477	A1	20030403	US 2002-215050	20020808

L73 ANSWER 18 OF 136 WPIDS (C) 2003 THOMSON DERWENT
TI Novel antiinflammatory peptide compounds comprising NEMO binding domain, useful for modulating NF-kappaB induction in a cell and for treating NF-kappaB-mediated inflammation disorders e.g., asthma, psoriasis, vasculitis.
PI US 2002156000 A1 20021024 (200320)* 47p A61K038-16
IN GHOSH, S; MAY, M J

L73 ANSWER 19 OF 136 MEDLINE DUPLICATE 14
TI Characterization of the Ikappa B-kinase NEMO binding domain.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2002 Nov 29) 277 (48) 45992-6000. Journal code: 2985121R. ISSN: 0021-9258.
AU May Michael J; Marienfeld Ralf B; Ghosh Sankar
AN 2002688173 MEDLINE

L73 ANSWER 20 OF 136 HCAPLUS COPYRIGHT 2003 ACS
TI IKKalpha , IKKbeta , and NEMO/IKKgamma Are Each Required for the NF-kappa B-mediated Inflammatory Response Program
SO Journal of Biological Chemistry (2002), 277(47), 45129-45140
CODEN: JBCHA3; ISSN: 0021-9258
AU Li, Xiang; Massa, Paul E.; Hanidu, Adedayo; Peet, Gregory W.; Aro, Patrick; Savitt, Ann; Mische, Sheenah; Li, Jun; Marcu, Kenneth B.
AN 2002:879607 HCAPLUS
DN 138:22609

L73 ANSWER 21 OF 136 MEDLINE DUPLICATE 15
TI The GTPase Rap1 regulates phorbol 12-myristate 13-acetate-stimulated but not ligand-induced beta 1 integrin-dependent leukocyte adhesion.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2002 Oct 25) 277 (43) 40893-900. Journal code: 2985121R. ISSN: 0021-9258.
AU Liu Li; Schwartz Barbara R; Tupper Joan; Lin Nancy; Winn Robert K; Harlan John M
AN 2002640703 MEDLINE

L73 ANSWER 22 OF 136 MEDLINE DUPLICATE 16
TI Association of the adaptor TANK with the I kappa B kinase (IKK) regulator NEMO connects IKK complexes with IKK epsilon and TBK1 kinases.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2002 Oct 4) 277 (40) 37029-36.
Journal code: 2985121R. ISSN: 0021-9258.
AU Chariot Alain; Leonardi Antonio; Muller Jurgen; Bonif Marianne; Brown Keith; Siebenlist Ulrich
AN 2002493273 MEDLINE

L73 ANSWER 23 OF 136 MEDLINE DUPLICATE 17
TI The death domain of NF-kappa B1 p105 is essential for signal-induced p105 proteolysis.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2002 Jul 5) 277 (27) 24162-8.
Journal code: 2985121R. ISSN: 0021-9258.
AU Beinke Soren; Belich Monica P; Ley Steven C
AN 2002348400 MEDLINE

L73 ANSWER 24 OF 136 MEDLINE DUPLICATE 18
TI NEMO trimerizes through its coiled-coil C-terminal domain.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2002 May 17) 277 (20) 17464-75.
Journal code: 2985121R. ISSN: 0021-9258.
AU Agou Fabrice; Ye Fei; Goffinont Stephane; Courtois Gilles; Yamaoka Shoji; Israel Alain; Veron Michel
AN 2002283355 MEDLINE

L73 ANSWER 25 OF 136 HCAPLUS COPYRIGHT 2003 ACS
TI Kinetic mechanisms of I.kappa.B-related kinases (IKK) inducible IKK and TBK-1 differ from IKK-1/IKK-2 heterodimer
SO Journal of Biological Chemistry (2002), 277(15), 12550-12558
CODEN: JBCHA3; ISSN: 0021-9258
AU Huynh, Q. Khai; Kishore, Nandini; Mathialagan, Sumathy; Donnelly, Ann M.; Tripp, Catherine S.
AN 2002:289984 HCAPLUS
DN 137:243804

L73 ANSWER 26 OF 136 MEDLINE DUPLICATE 19
TI The zinc finger domain of NEMO is selectively required for NF-kappa B activation by UV radiation and topoisomerase inhibitors.
SO MOLECULAR AND CELLULAR BIOLOGY, (2002 Aug) 22 (16) 5813-25.
Journal code: 8109087. ISSN: 0270-7306.
AU Huang Tony T; Feinberg Shelby L; Suryanarayanan Sainath; Miyamoto Shigeki
AN 2002389586 MEDLINE

L73 ANSWER 27 OF 136 MEDLINE DUPLICATE 20
TI Rap1 functions as a key regulator of T-cell and antigen-presenting cell interactions and modulates T-cell responses.
SO MOLECULAR AND CELLULAR BIOLOGY, (2002 Feb) 22 (4) 1001-15.
Journal code: 8109087. ISSN: 0270-7306.
AU Katagiri Koko; Hattori Masakazu; Minato Nagahiro; Kinashi Tatsuo
AN 2002083416 MEDLINE

L73 ANSWER 28 OF 136 MEDLINE DUPLICATE 21
TI SPAL, a Rap-specific GTPase activating protein, is present in the NMDA receptor-PSD-95 complex in the hippocampus.
SO GENES TO CELLS, (2002 Jun) 7 (6) 607-17.
Journal code: 9607379. ISSN: 1356-9597.
AU Roy Badal C; Kohu Kazuyoshi; Matsuura Ken; Yanai Hiroyuki; Akiyama Tetsu
AN 2002317482 MEDLINE

L73 ANSWER 29 OF 136 MEDLINE DUPLICATE 22
TI Stimulation of IKK-gamma oligomerization by the human T-cell leukemia virus oncoprotein Tax.
SO FEBS LETTERS, (2002 Nov 20) 531 (3) 494-8.

Journal code: 0155157. ISSN: 0014-5793.
AU Huang Guo Jin; Zhang Zhi Qing; Jin Dong Yan
AN 2002675175 MEDLINE

L73 ANSWER 30 OF 136 MEDLINE DUPLICATE 23
TI Anti-idiotypic protein domains selected from protein A-based affibody libraries.
SO PROTEINS, (2002 Aug 15) 48 (3) 454-62.
Journal code: 8700181. ISSN: 1097-0134.
AU Eklund Malin; Axelsson Lars; Uhlen Mathias; Nygren Per-Ake
AN 2002367154 MEDLINE

L73 ANSWER 31 OF 136 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Anti-idiotypic protein domains selected from protein A-based affibody libraries
SO PROTEINS-STRUCTURE FUNCTION AND GENETICS, (15 AUG 2002) Vol. 48, No. 3, pp. 454-462.
Publisher: WILEY-LISS, DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012 USA.
ISSN: 0887-3585.
AU Eklund M; Axelsson L; Uhlen M; Nygren P A (Reprint)
AN 2002:642296 SCISEARCH

L73 ANSWER 32 OF 136 MEDLINE DUPLICATE 24
TI Absence of inducible nitric oxide synthase modulates early reperfusion-induced NF-kappaB and AP-1 activation and enhances myocardial damage.
SO FASEB JOURNAL, (2002 Mar) 16 (3) 327-42.
Journal code: 8804484. ISSN: 1530-6860.
AU Zingarelli Basilia; Hake Paul W; Yang Zequan; O'Connor Michael; Denenberg Alvin; Wong Hector R
AN 2002144964 MEDLINE

L73 ANSWER 33 OF 136 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Absence of inducible nitric oxide synthase modulates early reperfusion-induced NF-kappa B and AP-1 activation and enhances myocardial damage
SO FASEB JOURNAL, (MAR 2002) Vol. 16, No. 3, pp. 327-342.
Publisher: FEDERATION AMER SOC EXP BIOL, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA.
ISSN: 0892-6638.
AU Zingarelli B (Reprint); Hake P W; Yang Z Q; O'Connor M; Denenberg A; Wong H R
AN 2002:315512 SCISEARCH

L73 ANSWER 34 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Regulation of macrophage cytokine and chemokine production by adipocyte fatty acid binding protein (aP2.
SO FASEB Journal, (March 20, 2002) Vol. 16, No. 4, pp. A319.
http://www.fasebj.org/. print.
Meeting Info.: Annual Meeting of the Professional Research Scientists on Experimental Biology New Orleans, Louisiana, USA April 20-24, 2002
ISSN: 0892-6638.
AU Brittingham, Katherine C. (1); Hotamisligil, Gokhan S.; Suttles, Jill (1)
AN 2002:353805 BIOSIS

L73 ANSWER 35 OF 136 MEDLINE DUPLICATE 25
TI Characterization of the bovine IkappaB kinases (IKK)alpha and IKKbeta, the regulatory subunit NEMO and their substrate IkappaBalpha.
SO GENE, (2002 Oct 16) 299 (1-2) 293-300.
Journal code: 7706761. ISSN: 0378-1119.
AU Rottenberg Sven; Schmuckli-Maurer Jacqueline; Grimm Stephan; Heussler Volker T; Dobbelaere Dirk A E
AN 2002698111 MEDLINE

L73 ANSWER 36 OF 136 MEDLINE DUPLICATE 26
TI Role of glycogen synthase kinase-3 in TNF-alpha-induced NF-kappaB activation and apoptosis in hepatocytes.
SO AMERICAN JOURNAL OF PHYSIOLOGY. GASTROINTESTINAL AND LIVER PHYSIOLOGY, (2002 Jul) 283 (1) G204-11.
Journal code: 100901227. ISSN: 0193-1857.
AU Schwabe Robert F; Brenner David A
AN 2002322322 MEDLINE

L73 ANSWER 37 OF 136 Elsevier BIOBASE COPYRIGHT 2003 Elsevier Science B.V.
AN 2002143265 ESBIOBASE
TI Role of glycogen synthase kinase-3 in TNF-.alpha.-induced NF-.kappa.B activation and apoptosis in hepatocytes
AU Schwabe R.F.; Brenner D.A.
CS D.A. Brenner, Univ. of North Carolina, Dept. of Medicine, CB #7038, Chapel Hill, NC 27599, United States.
E-mail: dab@med.unc.edu
SO American Journal of Physiology - Gastrointestinal and Liver Physiology, (2002), 283/1 46-1 (G204-G211), 47 reference(s)
CODEN: APGPDF ISSN: 0193-1857
DT Journal; Article
CY United States
LA English
SL English

L73 ANSWER 38 OF 136 HCAPLUS COPYRIGHT 2003 ACS
TI The role of IKK in constitutive activation of NF-.kappa.B transcription factor in prostate carcinoma cells
SO Journal of Cell Science (2002), 115(1), 141-151
CODEN: JNCSAI; ISSN: 0021-9533
AU Gasparian, Alexander V.; Yao, Ya Juan; Kowalczyk, Dariusz; Lyakh, Ludmila A.; Karseladze, Apollon; Slaga, Thomas J.; Budunova, Irina V.
AN 2002:88316 HCAPLUS
DN 136:260512

L73 ANSWER 39 OF 136 BIOTECHNO COPYRIGHT 2003 Elsevier Science B.V.
TI Development of a sensitive immunoradiometric assay for detection of platelet surface-associated immunoglobulins in thrombocytopenic dogs
SO American Journal of Veterinary Research, (01 JAN 2002), 63/1 (124-129), 19 reference(s)
CODEN: AJVRAH ISSN: 0002-9645
AU Scott M.A.; Kaiser L.; Davis J.M.; Schwartz K.A.
AN 2002.35100466 BIOTECHNO

L73 ANSWER 40 OF 136 MEDLINE DUPLICATE 27
TI A novel affinity gene fusion system allowing protein A-based recovery of non-immunoglobulin gene products.
SO JOURNAL OF BIOTECHNOLOGY, (2002 Oct 9) 99 (1) 41-50.
Journal code: 8411927. ISSN: 0168-1656.
AU Graslund Susanne; Eklund Malin; Falk Ronny; Uhlen Mathias; Nygren Per-Ake; Stahl Stefan
AN 2002448151 MEDLINE

L73 ANSWER 41 OF 136 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel isolated nucleic acid molecule encoding isolated IkB-kinase binding protein designated Y2H35, useful as probes and primers in molecular biology and biotechnology;
recombinant protein production via plasmid expression in host cell useful in gene therapy
AU Marcu K B
AN 2001-10472 BIOTECHDS
PI US 6214582 10 Apr 2001

L73 ANSWER 42 OF 136 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 29
TI Method based on IKK-.beta. interaction for identifying compounds for treatment of insulin resistance
SO PCT Int. Appl., 13 pp.
CODEN: PIXXD2
IN Shoelson, Steven
AN 2001:114936 HCAPLUS
DN 134:141753

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001010384	A2	20010215	WO 2000-US21805	20000810
WO 2001010384	A3	20010607		

PI
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 2000068987	A5	20010305	AU 2000-68987	20000810

L73 ANSWER 43 OF 136 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Modulating NF-kappaB induction in a cell, useful for treating e.g. inflammatory disorders, osteoporosis and cancer, comprises contacting a cell with an anti-inflammatory compound comprising at least one NEMO binding domain;
transcription factor induction, membrane translocation domain and NEMO binding peptide domain useful for disease therapy, diagnosis and drug screening
AU MAY M J; GHOSH S
AN 2002-09696 BIOTECHDS
PI WO 2001083547 8 Nov 2001

L73 ANSWER 44 OF 136 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel antiinflammatory compound comprising membrane translocation domain fused to NEMO binding sequence, useful for blocking nuclear factor kappaB activation, and for treating asthma, lung inflammation, psoriasis;
involving vector plasmid pBIIX-mediated gene transfer for expression in HeLa cell culture, for use in drug screening and therapy
AU MAY M J; GHOSH S; FINDEIS M A; PHILLIPS K
AN 2002-05632 BIOTECHDS
PI WO 2001083554 8 Nov 2001

L73 ANSWER 45 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Inhibitor of the inflammatory response induced by the TNFA and IL-1.
SO Official Gazette of the United States Patent and Trademark Office Patents, (July 24, 2001) Vol. 1248, No. 4, pp. No Pagination. e-file.
ISSN: 0098-1133.
AU Greene, Warner C.; Lin, Xin (1); Gelezuinas, Romas
AN 2001:447244 BIOSIS

L73 ANSWER 46 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI IkB kinase, subunits thereof, and methods of using same.
SO Official Gazette of the United States Patent and Trademark Office Patents, (June 5, 2001) Vol. 1247, No. 1, pp. No Pagination. e-file.
ISSN: 0098-1133.
AU Karin, Michael; DiDonato, Joseph A. (1); Rothwarf, David M.; Hayakawa, Makio; Zandi, Ebrahim
AN 2001:524963 BIOSIS

L73 ANSWER 47 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI IKK-alpha proteins, nucleic acids and methods.

SO Official Gazette of the United States Patent and Trademark Office Patents, (May 22, 2001) Vol. 1246, No. 4, pp. No Pagination. e-file. ISSN: 0098-1133.
AU Rothe, Mike; Cao, Zhaodan; Regnier, Catherine
AN 2001:514405 BIOSIS

L73 ANSWER 48 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI IKK-alpha proteins, nucleic acids and methods.
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AU Rothe, Mike; Cao, Zhaodan; Regnier, Catherine
AN 2001:514735 BIOSIS

L73 ANSWER 49 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI IKK-alpha proteins, nucleic acids and methods.
SO Official Gazette of the United States Patent and Trademark Office Patents, (May 22, 2001) Vol. 1246, No. 4, pp. No Pagination. e-file. ISSN: 0098-1133.
AU Rothe, Mike; Cao, Zhaodan (1); Regnier, Catherine
AN 2001:514732 BIOSIS

L73 ANSWER 50 OF 136 HCAPLUS COPYRIGHT 2003 ACS
TI IKK-.beta.-based method for identifying compounds for treatment of insulin resistance
SO U.S. Pat. Appl. Publ., 11 pp., Cont.-in-part of U.S. Ser. No. 636,150. CODEN: USXXCO
IN Shoelson, Steven
AN 2001:798710 HCAPLUS
DN 135:327359

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2001036625	A1	20011101	US 2001-776432	20010202
	US 6468755	B1	20021022	US 2000-636150	20000810
	US 2003044852	A1	20030306	US 2002-269553	20021011

L73 ANSWER 51 OF 136 WPIDS (C) 2003 THOMSON DERWENT
TI Novel antiinflammatory compound comprising membrane translocation domain fused to NEMO binding sequence, useful for blocking nuclear factor kappaB activation, and for treating asthma, lung inflammation, psoriasis.
PI WO 2001083554 A2 20011108 (200216)* EN 88p C07K014-705
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2001061164 A 20011112 (200222) C07K014-705
EP 1280820 A2 20030205 (200310) EN C07K014-08
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
IN FINDEIS, M A; GHOSH, S; MAY, M J; PHILLIPS, K

L73 ANSWER 52 OF 136 WPIDS (C) 2003 THOMSON DERWENT
TI Modulating NF-kappaB induction in a cell, useful for treating e.g. inflammatory disorders, osteoporosis and cancer, comprises contacting a cell with an anti-inflammatory compound comprising at least one NEMO binding domain.
PI WO 2001083547 A2 20011108 (200223)* EN 57p C07K014-47
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD

SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2001057631 A 20011112 (200223) C07K014-47
EP 1282643 A2 20030212 (200312) EN C07K014-47
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
IN GHOSH, S; MAY, M J

L73 ANSWER 53 OF 136 MEDLINE DUPLICATE 30
TI IKKgamma /NEMO facilitates the recruitment of the IkappaB proteins into the IkappaB kinase complex.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Sep 28) 276 (39) 36327-36.
Journal code: 2985121R. ISSN: 0021-9258.
AU Yamamoto Y; Kim D W; Kwak Y T; Prajapati S; Verma U; Gaynor R B
AN 2001522210 MEDLINE

L73 ANSWER 54 OF 136 MEDLINE DUPLICATE 31
TI Complete reconstitution of human IkappaB kinase (IKK) complex in yeast. Assessment of its stoichiometry and the role of IKKgamma on the complex activity in the absence of stimulation.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Sep 28) 276 (39) 36320-6.
Journal code: 2985121R. ISSN: 0021-9258.
AU Miller B S; Zandi E
AN 2001522209 MEDLINE

L73 ANSWER 55 OF 136 MEDLINE DUPLICATE 32
TI Effects of the NIK aly mutation on NF-kappaB activation by the Epstein-Barr virus latent infection membrane protein, lymphotoxin beta receptor, and CD40.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 May 4) 276 (18) 14602-6.
Journal code: 2985121R. ISSN: 0021-9258.
AU Luftig M A; Cahir-McFarland E; Mosialos G; Kieff E
AN 2001370772 MEDLINE

L73 ANSWER 56 OF 136 MEDLINE DUPLICATE 33
TI SIMPL is a tumor necrosis factor-specific regulator of nuclear factor-kappaB activity.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Mar 16) 276 (11) 7859-66.
Journal code: 2985121R. ISSN: 0021-9258.
AU Vig E; Green M; Liu Y; Yu K Y; Kwon H J; Tian J; Goebl M G; Harrington M A
AN 2001290680 MEDLINE

L73 ANSWER 57 OF 136 MEDLINE DUPLICATE 34
TI CD40 activates NF-kappa B and c-Jun N-terminal kinase and enhances chemokine secretion on activated human hepatic stellate cells.
SO JOURNAL OF IMMUNOLOGY, (2001 Jun 1) 166 (11) 6812-9.
Journal code: 2985117R. ISSN: 0022-1767.
AU Schwabe R F; Schnabl B; Kweon Y O; Brenner D A
AN 2001276277 MEDLINE

L73 ANSWER 58 OF 136 MEDLINE DUPLICATE 35
TI Protein kinase C-delta regulates thrombin-induced ICAM-1 gene expression in endothelial cells via activation of p38 mitogen-activated protein kinase.
SO MOLECULAR AND CELLULAR BIOLOGY, (2001 Aug) 21 (16) 5554-65.
Journal code: 8109087. ISSN: 0270-7306.
AU Rahman A; Anwar K N; Uddin S; Xu N; Ye R D; Platanias L C; Malik A B
AN 2001414571 MEDLINE

L73 ANSWER 59 OF 136 MEDLINE DUPLICATE 36
TI vCLAP, a caspase-recruitment domain-containing protein of equine Herpesvirus-2, persistently activates the Ikappa B kinases through oligomerization of IKKgamma.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Feb 2) 276 (5) 3183-7.
Journal code: 2985121R. ISSN: 0021-9258.

AU Poyet J L; Srinivasula S M; Alnemri E S
AN 2001269967 MEDLINE

L73 ANSWER 60 OF 136 MEDLINE DUPLICATE 37
TI Activation of the I kappa B alpha kinase (**IKK**) complex by double-stranded RNA-**binding** defective and catalytic inactive mutants of the interferon-inducible protein kinase PKR.
SO ONCOGENE, (2001 Apr 5) 20 (15) 1900-12.
Journal code: 8711562. ISSN: 0950-9232.
AU Ishii T; Kwon H; Hiscott J; Mosialos G; Koromilas A E
AN 2001237349 MEDLINE

L73 ANSWER 61 OF 136 MEDLINE DUPLICATE 38
TI Inhibitor of nuclear factor kappaB kinase beta is a key regulator of synovial inflammation.
SO ARTHRITIS AND RHEUMATISM, (2001 Aug) 44 (8) 1897-907.
Journal code: 0370605. ISSN: 0004-3591.
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AN 2001463547 MEDLINE

L73 ANSWER 62 OF 136 MEDLINE DUPLICATE 39
TI Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells.
SO JOURNAL OF EXPERIMENTAL MEDICINE, (2001 Dec 17) 194 (12) 1861-74.
Journal code: 2985109R. ISSN: 0022-1007.
AU Davis R E; Brown K D; Siebenlist U; Staudt L M
AN 2001700086 MEDLINE

L73 ANSWER 63 OF 136 MEDLINE DUPLICATE 40
TI NF-kappaB stimulates inducible nitric oxide synthase to protect mouse hepatocytes from TNF-alpha- and Fas-mediated apoptosis.
SO GASTROENTEROLOGY, (2001 Apr) 120 (5) 1251-62.
Journal code: 0374630. ISSN: 0016-5085.
AU Hatano E; Bennett B L; Manning A M; Qian T; Lemasters J J; Brenner D A
AN 2001169810 MEDLINE

L73 ANSWER 64 OF 136 MEDLINE DUPLICATE 41
TI Rap1 is involved in cell stretching modulation of p38 but not ERK or JNK MAP kinase.
SO JOURNAL OF CELL SCIENCE, (2001 Mar) 114 (Pt 6) 1221-7.
Journal code: 0052457. ISSN: 0021-9533.
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AN 2001258800 MEDLINE

L73 ANSWER 65 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Activation of NF-kappaB through the IKK signalsome is compromised in patients with a mutant form of IKKgamma/NEMO.
SO FASEB Journal, (March 8, 2001) Vol. 15, No. 5, pp. A1015. print.
Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001 Orlando, Florida, USA March 31-April 04, 2001
ISSN: 0892-6638.
AU Mahony, Alison O. (1); Elder, Melissa E.; Wara, Diane W.; Zonana, Jonathan; Greene, Warner C. (1)
AN 2001:314844 BIOSIS

L73 ANSWER 66 OF 136 MEDLINE DUPLICATE 42
TI Src family protein tyrosine kinase signaling mediates monosodium urate crystal-induced IL-8 expression by monocytic THP-1 cells.
SO JOURNAL OF LEUKOCYTE BIOLOGY, (2001 Dec) 70 (6) 961-8.
Journal code: 8405628. ISSN: 0741-5400.
AU Liu R; Aupperle K; Terkeltaub R

AN 2001692111 MEDLINE

L73 ANSWER 67 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Astrocyte:neutrophil interactions regulate MCP-1 and IL-8 expression during ischemia/reoxygenation.
SO Society for Neuroscience Abstracts, (2001) Vol. 27, No. 1, pp. 882. print.
Meeting Info.: 31st Annual Meeting of the Society for Neuroscience San Diego, California, USA November 10-15, 2001
ISSN: 0190-5295.
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AN 2001:509464 BIOSIS

L73 ANSWER 68 OF 136 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Molecular cloning and characterizaiton of a novel **IKK**-kinase (NAK) **binding** protein (NAKBP) which inhibits NF-kappa B activation.
SO GASTROENTEROLOGY, (APR 2001) Vol. 120, No. 5, Supp. [1], pp. A497-A497. MA 2529.
Publisher: W B SAUNDERS CO, INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399 USA.
ISSN: 0016-5085.
AU Fujita F (Reprint); Joh T; Seno K; Matsui T; Okumura F; Kataoka H; Sasaki M; Ohshima T; Takezono Y; Yokoyama Y; Itoh M; Nakanishi M
AN 2001:500372 SCISEARCH

L73 ANSWER 69 OF 136 MEDLINE DUPLICATE 43
TI Mechanisms of ubiquitin-mediated, limited processing of the NF-kappaB1 precursor protein p105.
SO BIOCHIMIE, (2001 Mar-Apr) 83 (3-4) 341-9.
Journal code: 1264604. ISSN: 0300-9084.
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AN 2001341030 MEDLINE

L73 ANSWER 70 OF 136 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 44
TI The sesquiterpene lactone parthenolide **binds** and inhibits **IKK** beta
SO MOLECULAR BIOLOGY OF THE CELL, (NOV 2001) Vol. 12, Supp. [S], pp. 271A-271A. MA 1479.
Publisher: AMER SOC CELL BIOLOGY, 8120 WOODMONT AVE, STE 750, BETHESDA, MD 20814-2755 USA.
ISSN: 1059-1524.
AU Kwok B H (Reprint); Koh B D; Ndubuisi M I; Elofsson M; Crews C M
AN 2002:40958 SCISEARCH

L73 ANSWER 71 OF 136 MEDLINE DUPLICATE 45
TI Zinc activates NF-kappaB in HUT-78 cells.
SO JOURNAL OF LABORATORY AND CLINICAL MEDICINE, (2001 Oct) 138 (4) 250-6.
Journal code: 0375375. ISSN: 0022-2143.
AU Prasad A S; Bao B; Beck F W; Sarkar F H
AN 2001542940 MEDLINE

L73 ANSWER 72 OF 136 MEDLINE DUPLICATE 46
TI CD98 induces LFA-1-mediated cell adhesion in lymphoid cells via activation of Rap1.
SO FEBS LETTERS, (2001 Feb 2) 489 (2-3) 249-53.
Journal code: 0155157. ISSN: 0014-5793.
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AN 2001151862 MEDLINE

L73 ANSWER 73 OF 136 HCAPLUS COPYRIGHT 2003 ACS
TI Cell-cell interactions and abnormality in immune and hematopoietic systems. The role of Rap 1G protein
SO Immunology Frontier (2001), 11(4), 217-225

CODEN: IMFREG; ISSN: 0917-0774
AU Minato, Nagahiro
AN 2001:645887 HCAPLUS
DN 135:209458

L73 ANSWER 74 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Protein kinase C-delta regulates thrombin-induced ICAM-1 gene transcription in endothelial cells via activation of p38 MAP kinase.
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Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001 Orlando, Florida, USA March 31-April 04, 2001
ISSN: 0892-6638.
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AN 2001:257582 BIOSIS

L73 ANSWER 75 OF 136 MEDLINE DUPLICATE 47
TI Functional redundancy of the zinc fingers of A20 for inhibition of NF-kappaB activation and protein-protein interactions.
SO FEBS LETTERS, (2001 Jun 1) 498 (1) 93-7.
Journal code: 0155157. ISSN: 0014-5793.
AU Klinkenberg M; Van Huffel S; Heyninck K; Beyaert R
AN 2001319853 MEDLINE

L73 ANSWER 76 OF 136 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 48
TI Differential role of I kappa B kinase 1 and 2 in primary rat hepatocytes
SO HEPATOLOGY, (JAN 2001) Vol. 33, No. 1, pp. 81-90.
Publisher: W B SAUNDERS CO, INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399 USA.
ISSN: 0270-9139.
AU Schwabe R F; Bennett B L; Manning A M; Brenner D A (Reprint)
AN 2001:37145 SCISEARCH

L73 ANSWER 77 OF 136 MEDLINE DUPLICATE 49
TI Multiple signaling pathways regulate NF-kappaB-dependent transcription of the monocyte chemoattractant protein-1 gene in primary endothelial cells.
SO BLOOD, (2001 Jan 1) 97 (1) 46-55.
Journal code: 7603509. ISSN: 0006-4971.
AU Goebeler M; Gillitzer R; Kilian K; Utzel K; Brocker E B; Rapp U R; Ludwig S
AN 2001117436 MEDLINE

L73 ANSWER 78 OF 136 LIFESCI COPYRIGHT 2003 CSA
TI I Kappa B kinase, subunits thereof, and methods of using same
SO (20010605) . US Patent: 6242253; US CLASS: 435/325; 435/194; 435/252.3; 435/320.1; 536/23.2.
AU Karin, M.; DiDonato, J.A.; Rothwarf, D.M.; Hayakawa, M.; Zandi, E.
AN 2002:9310 LIFESCI

L73 ANSWER 79 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Molecular cloning and characterization of a novel **IKK**-kinase (NAK) **binding** protein (NAKBP) which inhibits NF-kappaB activation.
SO Gastroenterology, (April, 2001) Vol. 120, No. 5 Supplement 1, pp. A.497. http://www.gastrojournal.org/. print.
Meeting Info.: 102nd Annual Meeting of the American Gastroenterological Association and Digestive Disease Week Atlanta, Georgia, USA May 20-23, 2001
ISSN: 0016-5085.
AU Fujita, Fumitaka (1); Joh, Takashi; Seno, Kyoji (1); Matsui, Taido (1); Okumura, Fuminori (1); Kataoka, Hiromi (1); Sasaki, Makoto (1); Ohshima, Tadayuki (1); Takezono, Yasuhide (1); Yokoyama, Yoshifumi (1); Itoh, Makoto (1); Nakanishi, Makoto (1)

AN 2002:201052 BIOSIS

L73 ANSWER 80 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 50
TI Y2H56 A strong **IKK binding** protein.
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ISSN: 0098-1133.
AU Marcu, Kenneth B. (1)
AN 2001:12520 BIOSIS

L73 ANSWER 81 OF 136 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New isolated I kappa-B-kinase (IKK) and nucleic acids encoding IKK, useful for detecting IKK complexes and modulating IKK activity in cells undergoing signaling by inflammatory mediators, especially useful for controlling inflammation;
recombinant protein production in host cell for antiinflammatory and immunosuppressive activity
AU Marcu K B
AN 2000-11099 BIOTECHDS
PI US 6066474 23 May 2000

L73 ANSWER 82 OF 136 MEDLINE DUPLICATE 51
TI Inhibition of NF-kappa B activation by arsenite through reaction with a critical cysteine in the activation loop of Ikappa B kinase.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Nov 17) 275 (46) 36062-6.
Journal code: 2985121R. ISSN: 0021-9258.
AU Kapahi P; Takahashi T; Natoli G; Adams S R; Chen Y; Tsien R Y; Karin M
AN 2001074348 MEDLINE

L73 ANSWER 83 OF 136 MEDLINE DUPLICATE 52
TI Domain-specific interaction with the I kappa B kinase (IKK)regulatory subunit IKK gamma is an essential step in tax-mediated activation of IKK.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Nov 3) 275 (44) 34060-7.
Journal code: 2985121R. ISSN: 0021-9258.
AU Xiao G; Harhaj E W; Sun S C
AN 2001048342 MEDLINE

L73 ANSWER 84 OF 136 HCAPLUS COPYRIGHT 2003 ACS
TI Src homology domain 2-containing tyrosine phosphatase 2 associates with intercellular adhesion molecule 1 to regulate cell survival
SO Journal of Biological Chemistry (2000), 275(39), 30029-30036
CODEN: JBCHA3; ISSN: 0021-9258
AU Pluskota, Elzbieta; Chen, Yiming; D'Souza, Stanley E.
AN 2000:722282 HCAPLUS
DN 133:348107

L73 ANSWER 85 OF 136 MEDLINE DUPLICATE 53
TI An induced proximity model for NF-kappa B activation in the Nod1/RICK and RIP signaling pathways.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Sep 8) 275 (36) 27823-31.
Journal code: 2985121R. ISSN: 0021-9258.
AU Inohara N; Koseki T; Lin J; del Peso L; Lucas P C; Chen F F; Ogura Y; Nunez G
AN 2000496005 MEDLINE

L73 ANSWER 86 OF 136 MEDLINE DUPLICATE 54
TI Inhibition of IkappaB kinase activity by sodium salicylate in vitro does not reflect its inhibitory mechanism in intact cells.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Apr 14) 275 (15) 10925-9.
Journal code: 2985121R. ISSN: 0021-9258.
AU Alpert D; Vilcek J
AN 2000219131 MEDLINE

L73 ANSWER 87 OF 136 MEDLINE DUPLICATE 55
TI Rap2 as a slowly responding molecular switch in the Rap1 signaling cascade.
SO MOLECULAR AND CELLULAR BIOLOGY, (2000 Aug) 20 (16) 6074-83.
Journal code: 8109087. ISSN: 0270-7306.
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AN 2000414769 MEDLINE

L73 ANSWER 88 OF 136 MEDLINE DUPLICATE 56
TI Activation of IKKalpha and IKKbeta through their fusion with HTLV-I tax protein.
SO ONCOGENE, (2000 Oct 26) 19 (45) 5198-203.
Journal code: 8711562. ISSN: 0950-9232.
AU Xiao G; Sun S C
AN 2000512052 MEDLINE

L73 ANSWER 89 OF 136 HCAPLUS COPYRIGHT 2003 ACS
TI Mixed-lineage kinase 3 delivers CD3/CD28-derived signals into the I.kappa.B kinase complex
SO Molecular and Cellular Biology (2000), 20(7), 2556-2568
CODEN: MCEBD4; ISSN: 0270-7306
AU Hehner, Steffen P.; Hofmann, Thomas G.; Ushmorov, Alexej; Dienz, Oliver; Leung, Irene Wing-Lan; Lassam, Norman; Scheidereit, Claus; Droge, Wulf; Schmitz, M. Lienhard
AN 2000:193394 HCAPLUS
DN 133:54457

L73 ANSWER 90 OF 136 MEDLINE DUPLICATE 57
TI Selective inhibition of NF-kappaB activation by a peptide that blocks the interaction of NEMO with the IkappaB kinase complex.
SO SCIENCE, (2000 Sep 1) 289 (5484) 1550-4.
Journal code: 0404511. ISSN: 0036-8075.
AU May M J; D'Acquisto F; Madge L A; Glockner J; Pober J S; Ghosh S
AN 2000431571 MEDLINE

L73 ANSWER 91 OF 136 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Downregulation of **IKK** activity and NF-kappa B **binding** activity is important for the acquisition of a polyploid state in megakaryocyte.
SO FASEB JOURNAL, (11 MAY 2000) Vol. 14, No. 8, pp. 1412-1412.
Publisher: FEDERATION AMER SOC EXP BIOL, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998.
ISSN: 0892-6638.
AU Zhang Y (Reprint); Sun S S; Wang Z G; Zimmet J; Kaluzhny Y; Ravid K
AN 2000:560164 SCISEARCH

L73 ANSWER 92 OF 136 HCAPLUS COPYRIGHT 2003 ACS
TI Severe liver degeneration and lack of NF-.kappa.B activation in NEMO/IKK .gamma.-deficient mice
SO Genes & Development (2000), 14(7), 854-862
CODEN: GEDEEP; ISSN: 0890-9369
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AN 2000:298007 HCAPLUS
DN 133:203742

L73 ANSWER 93 OF 136 MEDLINE DUPLICATE 58
TI Recruitment of the IKK signalosome to the p55 TNF receptor: RIP and A20 **bind** to NEMO (**IKKgamma**) upon receptor stimulation.
SO IMMUNITY, (2000 Mar) 12 (3) 301-11.
Journal code: 9432918. ISSN: 1074-7613.
AU Zhang S Q; Kovalenko A; Cantarella G; Wallach D
AN 2000216393 MEDLINE

L73 ANSWER 94 OF 136 HCAPLUS COPYRIGHT 2003 ACS
TI Oncogenic viruses and ubiquitin-proteasome system
SO Molecular Medicine (Tokyo) (2000), 37(2), 144-150
CODEN: MOLMEL; ISSN: 0918-6557
AU Nakajima, Takuma
AN 2000:94334 HCAPLUS
DN 133:55735

L73 ANSWER 95 OF 136 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Newly isolated human kinase, IkappaB-kinase (IKK-alpha) polypeptides; expression in host cell, DNA probe, DNA primer and antibody, used for diagnosis and therapy
AU Rothe M; Cao Z; Regnier C
AN 1999-04021 BIOTECHDS
PI WO 9901541 14 Jan 1999

L73 ANSWER 96 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 60
TI Y2H61 an **IKK binding** protein.
SO Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 26, 1999) Vol. 1227, No. 4, pp. No pagination. e-file.
ISSN: 0098-1133.
AU Marcu, Kenneth B. (1)
AN 2000:277590 BIOSIS

L73 ANSWER 97 OF 136 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 61
TI Human I.kappa.B kinase .beta. subunit (IKK.beta.), its cDNA sequences, recombinant expression, and use in treating inflammation and in identifying anti-inflammatory drugs
SO PCT Int. Appl., 46 pp.
CODEN: PIXXD2
IN Chu, Keting; Pot, David
AN 1999:464103 HCAPLUS
DN 131:84843

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9934000	A1	19990708	WO 1998-US27917	19981230
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6030834	A	20000229	US 1998-215131	19981218
AU 9920242	A1	19990719	AU 1999-20242	19981230

L73 ANSWER 98 OF 136 HCAPLUS COPYRIGHT 2003 ACS
TI A novel inhibitor of the inflammatory response induced by TNF.alpha. and IL-1
SO PCT Int. Appl., 48 pp.
CODEN: PIXXD2
IN Greene, Warner C.; Lin, Xin; Gelezuinas, Romas
AN 1999:566073 HCAPLUS
DN 131:198629

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943704	A1	19990902	WO 1999-US4110	19990225
W: AU, CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9928778	A1	19990915	AU 1999-28778	19990225

US 6265538	B1	20010724	US 1999-257703	19990225
US 2002042499	A1	20020411	US 2001-871889	20010601

L73 ANSWER 99 OF 136 HCAPLUS COPYRIGHT 2003 ACS
TI Cloning and expression of cDNA for human low-molecular-weight G protein-activating **Spa-1** protein
SO PCT Int. Appl., 80 pp.
CODEN: PIXXD2
IN Minato, Nagahiro
AN 1999:166645 HCAPLUS
DN 130:192794

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9910380	A1	19990304	WO 1998-JP3715	19980821

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9887488	A1	19990316	AU 1998-87488	19980821
JP 11137283	A2	19990525	JP 1998-250344	19980821

L73 ANSWER 100 OF 136 WPIDS (C) 2003 THOMSON DERWENT
TI Screening for agents which modulate the interaction of **IKK**-beta polypeptides and their **binding** targets.
PI US 5916760 A 19990629 (199932)* 14p C12P001-48
IN GOEDDEL, D V; WORONICZ, J

L73 ANSWER 101 OF 136 MEDLINE DUPLICATE 62
TI Heat shock inhibits radiation-induced activation of NF-kappaB via inhibition of I-kappaB kinase.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Aug 13) 274 (33) 23061-7.
Journal code: 2985121R. ISSN: 0021-9258.
AU Curry H A; Clemens R A; Shah S; Bradbury C M; Botero A; Goswami P; Gius D
AN 1999367428 MEDLINE

L73 ANSWER 102 OF 136 MEDLINE DUPLICATE 63
TI IKKgamma serves as a docking subunit of the IkappaB kinase (IKK) and mediates interaction of IKK with the human T-cell leukemia virus Tax protein.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Aug 13) 274 (33) 22911-4.
Journal code: 2985121R. ISSN: 0021-9258.
AU Harhaj E W; Sun S C
AN 1999367408 MEDLINE

L73 ANSWER 103 OF 136 MEDLINE DUPLICATE 64
TI Rap1 GTPase-activating protein **SPA-1** negatively regulates cell adhesion.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Jun 25) 274 (26) 18463-9.
Journal code: 2985121R. ISSN: 0021-9258.
AU Tsukamoto N; Hattori M; Yang H; Bos J L; Minato N
AN 1999303611 MEDLINE

L73 ANSWER 104 OF 136 MEDLINE DUPLICATE 65
TI Role of adapter function in oncoprotein-mediated activation of NF-kappaB. Human T-cell leukemia virus type I Tax interacts directly with IkappaB kinase gamma.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Jun 18) 274 (25) 17402-5.
Journal code: 2985121R. ISSN: 0021-9258.
AU Jin D Y; Giordano V; Kibler K V; Nakano H; Jeang K T
AN 1999292691 MEDLINE

L73 ANSWER 105 OF 136 MEDLINE DUPLICATE 66
TI NF-kappaB activation by a signaling complex containing TRAF2, TANK and TBK1, a novel IKK-related kinase.
SO EMBO JOURNAL, (1999 Dec 1) 18 (23) 6694-704.
Journal code: 8208664. ISSN: 0261-4189.
AU Pomerantz J L; Baltimore D
AN 2000050564 MEDLINE

L73 ANSWER 106 OF 136 MEDLINE DUPLICATE 67
TI The kinetics of association and phosphorylation of IkappaB isoforms by IkappaB kinase 2 correlate with their cellular regulation in human endothelial cells.
SO BIOCHEMISTRY, (1999 May 11) 38 (19) 6231-8.
Journal code: 0370623. ISSN: 0006-2960.
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AN 1999255380 MEDLINE

L73 ANSWER 107 OF 136 MEDLINE DUPLICATE 68
TI The antiinflammatory sesquiterpene lactone parthenolide inhibits NF-kappa B by targeting the I kappa B kinase complex.
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Journal code: 2985117R. ISSN: 0022-1767.
AU Hehner S P; Hofmann T G; Droge W; Schmitz M L
AN 2000021862 MEDLINE

L73 ANSWER 108 OF 136 MEDLINE DUPLICATE 69
TI Activation of IkappaB kinase beta by protein kinase C isoforms.
SO MOLECULAR AND CELLULAR BIOLOGY, (1999 Mar) 19 (3) 2180-8.
Journal code: 8109087. ISSN: 0270-7306.
AU Lallena M J; Diaz-Meco M T; Bren G; Paya C V; Moscat J
AN 1999147052 MEDLINE

L73 ANSWER 109 OF 136 MEDLINE DUPLICATE 70
TI IkappaB kinase (IKK)-associated protein 1, a common component of the heterogeneous IKK complex.
SO MOLECULAR AND CELLULAR BIOLOGY, (1999 Feb) 19 (2) 1526-38.
Journal code: 8109087. ISSN: 0270-7306.
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AN 1999108125 MEDLINE

L73 ANSWER 110 OF 136 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Downregulation of **IKK** activity and NF-kappa B **binding** activity during the acquisition of a polyploid state in megakaryocytes.
SO BLOOD, (15 NOV 1999) Vol. 94, No. 10, Part 1, Supp. [1], pp. 1209-1209.
Publisher: AMER SOC HEMATOLOGY, 1200 19TH ST, NW, STE 300, WASHINGTON, DC 20036-2422.
ISSN: 0006-4971.
AU Zhang Y (Reprint); Sun S; Wang Z; Zimmet J; Ravid K
AN 2000:52334 SCISEARCH

L73 ANSWER 111 OF 136 MEDLINE DUPLICATE 71
TI The E6 oncoproteins of high-risk papillomaviruses **bind** to a novel putative GAP protein, E6TP1, and target it for degradation.
SO MOLECULAR AND CELLULAR BIOLOGY, (1999 Jan) 19 (1) 733-44.
Journal code: 8109087. ISSN: 0270-7306.
AU Gao Q; Srinivasan S; Boyer S N; Wazer D E; Band V
AN 1999078010 MEDLINE

L73 ANSWER 112 OF 136 MEDLINE DUPLICATE 72
TI Recruitment of a ROC1-CUL1 ubiquitin ligase by Skp1 and HOS to catalyze the ubiquitination of I kappa B alpha.

SO MOLECULAR CELL, (1999 Apr) 3 (4) 527-33.
Journal code: 9802571. ISSN: 1097-2765.
AU Tan P; Fuchs S Y; Chen A; Wu K; Gomez C; Ronai Z; Pan Z Q
AN 1999247021 MEDLINE

L73 ANSWER 113 OF 136 HCAPLUS COPYRIGHT 2003 ACS
TI The new target of aspirin. IKK.beta.
SO Jikken Igaku (1999), 17(4), 487-489
CODEN: JIIGEF; ISSN: 0288-5514
AU Yamamoto, Yumi; Yin, Min Jean; Gaynor, Richard B.
AN 1999:136939 HCAPLUS
DN 130:177025

L73 ANSWER 114 OF 136 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 73
TI Activation of nuclear factor kappa B as a target for anti-inflammatory therapy
SO GUT, (MAR 1999) Vol. 44, No. 3, pp. 309-310.
Publisher: BRITISH MED JOURNAL PUBL GROUP, BRITISH MED ASSOC HOUSE, TAVISTOCK SQUARE, LONDON WC1H 9JR, ENGLAND.
ISSN: 0017-5749.
AU Schreiber S (Reprint)
AN 1999:188265 SCISEARCH

L73 ANSWER 115 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Downregulation of **IKK** activity and NF-kappaB **binding** activity during the acquisition of a polyploid state in megakaryocytes.
SO Blood, (Nov. 15, 1999) Vol. 94, No. 10 SUPPL. 1 PART 1, pp. 271a.
Meeting Info.: Forty-first Annual Meeting of the American Society of Hematology New Orleans, Louisiana, USA December 3-7, 1999 The American Society of Hematology
. ISSN: 0006-4971.
AU Zhang, Ying (1); Sun, Shishinn (1); Wang, Zhengyu (1); Zimmet, Jeffrey (1); Ravid, Katya (1)
AN 2000:46064 BIOSIS

L73 ANSWER 116 OF 136 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Downregulation of **IKK** activity and NF-kappa B **binding** activity during the acquisition of a polyploid state in megakaryocytes
SO MOLECULAR BIOLOGY OF THE CELL, (NOV 1999) Vol. 10, Supp. [S], pp. 268-268.
Publisher: AMER SOC CELL BIOLOGY, PUBL OFFICE, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 1059-1524.
AU Zhang Y (Reprint); Sun S; Wang Z Y; Zimmet J; Ravid K
AN 1999:979715 SCISEARCH

L73 ANSWER 117 OF 136 HCAPLUS COPYRIGHT 2003 ACS
TI Design and synthesis of novel functional analogs of spongistatin as anti-cancer agents
SO Book of Abstracts, 217th ACS National Meeting, Anaheim, Calif., March 21-25 (1999), MEDI-240 Publisher: American Chemical Society, Washington, D. C.
CODEN: 67GHA6
AU Mao, C.; Huang, H.; Jan, S.-T.; Navara, C.; Narla, R. K.; Uckun, F. M.
AN 1999:92754 HCAPLUS

L73 ANSWER 118 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Downregulation of **IKK** activity and NF-kappaB **binding** activity during the acquisition of a polyploid state in megakaryocytes.
SO Molecular Biology of the Cell, (Nov., 1999) Vol. 10, No. SUPPL., pp. 46a.
Meeting Info.: 39th Annual Meeting of the American Society for Cell Biology Washington, D.C., USA December 11-15, 1999 The American Society for Cell Biology
. ISSN 1059-1524.
AU Zhang, Ying (1); Sun, Shishinn (1); Wang, Zhengyu (1); Zimmet, Jeffrey

(1); Ravid, Katya (1)
AN 2000:27662 BIOSIS

L73 ANSWER 119 OF 136 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 74
TI I.kappa.b kinase, its subunits and sequences, and methods for their use
SO PCT Int. Appl., 102 pp.
CODEN: PIXXD2
IN Karin, Michael; Didonato, Joseph A.; Rothwarf, David M.; Hayakawa, Makio; Zandi, Ebrahim
AN 1998:605030 HCAPLUS
DN 129:213855

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9837228	A1	19980827	WO 1998-US3511	19980223
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 6268194	B1	20010731	US 1997-810131	19970225
AU 9866646	A1	19980909	AU 1998-66646	19980223
AU 740622	B2	20011108		
EP 981642	A1	20000301	EP 1998-908673	19980223
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001524813	T2	20011204	JP 1998-536953	19980223
US 6242253	B1	20010605	US 1998-168629	19981008
US 2002045235	A1	20020418	US 2001-796872	20010228

L73 ANSWER 120 OF 136 HCAPLUS COPYRIGHT 2003 ACS
TI Cloning of cDNA and genomic DNA for a novel nuclear **SPA-1** protein that regulates cell cycle
SO U.S., 31 pp., Cont.-in-part of U.S. Ser. No. 325,909, abandoned.
CODEN: USXXAM
IN Minato, Nagahiro; Hattori, Masakazu; Kubota, Hiroshi; Maeda, Masatsugu
AN 1998:719169 HCAPLUS
DN 129:326979

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 5831024	A	19981103	US 1995-380403	19950130
US 5998585	A	19991207	US 1997-895628	19970717
US 6406886	B1	20020618	US 1997-895810	19970717

L73 ANSWER 121 OF 136 WPIDS (C) 2003 THOMSON DERWENT
TI New isolated stimulus-inducible I-kappa-B kinase signalsome - useful for developing products for treating, e.g. inflammatory neuro-degenerative and auto-immune diseases.

PI WO 9808955	A1	19980305 (199816)*	EN	115p	C12N015-54
RW: AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
W: AU CA JP					
AU 9740904	A	19980319 (199831)			C12N015-54
EP 920518	A1	19990609 (199927)	EN		C12N015-54
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
US 5972674	A	19991026 (199952)			C12N009-12
AU 726383	B	20001102 (200062)			C12N015-54
JP 2001502892	W	20010306 (200116)		106p	C12N015-09
US 6258579	B1	20010710 (200141)			C12N009-12
US 2002151021	A1	20021017 (200270)			C12N009-12

IN BARBOSA, M; LI, G; MERCURIO, F; MURRAY, B W; ZHU, H; LI, J W

L73 ANSWER 122 OF 136 MEDLINE DUPLICATE 75
TI The tax oncoprotein of human T-cell leukemia virus type 1 associates with and persistently activates IkappaB kinases containing IKKalpha and IKKbeta.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jun 26) 273 (26) 15891-4.
Journal code: 2985121R. ISSN: 0021-9258.
AU Chu Z L; DiDonato J A; Hawiger J; Ballard D W

AN 1998298086 MEDLINE

L73 ANSWER 123 OF 136 MEDLINE DUPLICATE 76
TI IKK-gamma is an essential regulatory subunit of the IkappaB kinase complex.
SO NATURE, (1998 Sep 17) 395 (6699) 297-300.
Journal code: 0410462. ISSN: 0028-0836.
AU Rothwarf D M; Zandi E; Natoli G; Karin M
AN 1998421680 MEDLINE

L73 ANSWER 124 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI IKK-gamma is an essential regulatory subunit of the IkappaB kinase complex.
SO Nature (London), (Sept. 17, 1998) Vol. 39, No. 6699, pp. 297-300.
ISSN: 0028-0836.
AU Rothwarf, David M.; Zandi, Ebrahim; Natoll, Gioacchino; Karin, Michaelo (1)
AN 1998:446874 BIOSIS

L73 ANSWER 125 OF 136 MEDLINE DUPLICATE 77
TI IKAP is a scaffold protein of the IkappaB kinase complex.
SO NATURE, (1998 Sep 17) 395 (6699) 292-6.
Journal code: 0410462. ISSN: 0028-0836.
AU Cohen L; Henzel W J; Baeuerle P A
AN 1998421679 MEDLINE

L73 ANSWER 126 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI IKAP is a scaffold protein of the IkappaB kinase complex.
SO Nature (London), (Sept. 17, 1998) Vol. 39, No. 6699, pp. 292-296.
ISSN: 0028-0836.
AU Cohen, Lucie; Henzel, William J.; Baeuerle, Patrick A. (1)
AN 1998:446873 BIOSIS

L73 ANSWER 127 OF 136 MEDLINE DUPLICATE 78
TI The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta.
SO NATURE, (1998 Nov 5) 396 (6706) 77-80.
Journal code: 0410462. ISSN: 0028-0836.
AU Yin M J; Yamamoto Y; Gaynor R B
AN 1999032115 MEDLINE

L73 ANSWER 128 OF 136 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI A study of the interactions between an IgG-**binding** domain based on the B domain of staphylococcal protein A and rabbit IgC
SO MOLECULAR BIOTECHNOLOGY, (AUG 1998) Vol. 10, No. 1, pp. 9-16.
Publisher: HUMANA PRESS INC, 999 RIVERVIEW DRIVE SUITE 208, TOTOWA, NJ 07512.
ISSN: 1073-6085.
AU Brown N L (Reprint); Bottomley S P; Scawen M D; Gore M G
AN 1998:686291 SCISEARCH

L73 ANSWER 129 OF 136 MEDLINE DUPLICATE 79
TI Human **SPA-1** gene product selectively expressed in lymphoid tissues is a specific GTPase-activating protein for Rap1 and Rap2. Segregate expression profiles from a rap1GAP gene product.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Oct 31) 272 (44) 28081-8.
Journal code: 2985121R. ISSN: 0021-9258.
AU Kurachi H; Wada Y; Tsukamoto N; Maeda M; Kubota H; Hattori M; Iwai K; Minato N
AN 1998010656 MEDLINE

L73 ANSWER 130 OF 136 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 80
TI Cryptic origin of SPAI, a plasma protein with a transglutaminase substrate domain and the WAP motif, revealed by in situ hybridization and

immunohistochemistry.
SO Journal of Biological Chemistry, (1996) 271/47 (29517-29520).
ISSN: 0021-9258 CODEN: JBCHA3
AU Furukawa M.; Suzuki Y.; Ghoneim M.A.; Tachibana S.; Hirose S.
AN 96354878 EMBASE

L73 ANSWER 131 OF 136 MEDLINE DUPLICATE 81
TI Overexpression and functional analysis of a mitogen-inducible nuclear GTPase activating protein, **Spa-1**.
SO INTERNATIONAL JOURNAL OF BIOCHEMISTRY AND CELL BIOLOGY, (1996 Nov) 28 (11) 1241-7.
Journal code: 9508482. ISSN: 1357-2725.
AU Nur-e-Kamal M S
AN 97174555 MEDLINE

L73 ANSWER 132 OF 136 MEDLINE DUPLICATE 82
TI Molecular cloning of a novel mitogen-inducible nuclear protein with a Ran GTPase-activating domain that affects cell cycle progression.
SO MOLECULAR AND CELLULAR BIOLOGY, (1995 Jan) 15 (1) 552-60.
Journal code: 8109087. ISSN: 0270-7306.
AU Hattori M; Tsukamoto N; Nur-e-Kamal M S; Rubinfeld B; Iwai K; Kubota H; Maruta H; Minato N
AN 95098034 MEDLINE

L73 ANSWER 133 OF 136 HCAPLUS COPYRIGHT 2003 ACS
TI Reproducible preparation of catalysts for methacrylic acid synthesis
SO Jpn. Kokai Tokkyo Koho, 13 pp.
CODEN: JKXXAF
IN Ishii, Toru; Aoki, Yukio; Wada, Masahiro
AN 1989:232268 HCAPLUS
DN 110:232268

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 63315148	A2	19881222	JP 1987-150111	19870618
	JP 06079666	B4	19941012		

L73 ANSWER 134 OF 136 MEDLINE DUPLICATE 83
TI Characterization of complexes containing protein A and rabbit immunoglobulin G or Fc gamma fragments.
SO RESEARCH COMMUNICATIONS IN CHEMICAL PATHOLOGY AND PHARMACOLOGY, (1986 Jan) 51 (1) 117-28.
Journal code: 0244734. ISSN: 0034-5164.
AU Das C; Shearer W T; Langone J J
AN 86150821 MEDLINE

L73 ANSWER 135 OF 136 MEDLINE DUPLICATE 84
TI Effect of protein A and its fragment B on the catabolic and Fc receptor sites of IgG.
SO EUROPEAN JOURNAL OF IMMUNOLOGY, (1983 Aug) 13 (8) 605-14.
Journal code: 1273201. ISSN: 0014-2980.
AU Dima S; Medesan C; Mota G; Moraru I; Sjoquist J; Ghetie V
AN 83287561 MEDLINE

L73 ANSWER 136 OF 136 MEDLINE
TI [Health and nursing care are not merely a good combination].
Omsorg og pleje er **ikke** kun fine for-**bindinger**.
SO SYGEPLEJERSKEN, (1978 May 10) 78 (18) 9, 22.
Journal code: 0421366. ISSN: 0106-8350.
AU Tegner R
AN 78181119 MEDLINE

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=> d ab 38,42,54,68,79,93,99,105

L73 ANSWER 38 OF 136 HCAPLUS COPYRIGHT 2003 ACS
AB Rel/NF-.kappa.B transcription factors are implicated in the control of cell proliferation, apoptosis and transformation. The key to NF-.kappa.B regulation is the inhibitory I.kappa.B proteins. During response to diverse stimuli, I.kappa.Bs are rapidly phosphorylated by I.kappa.B kinases (IKKs), ubiquitinated and undergo degrdn. The authors have investigated the expression and function of NF-.kappa.B, I.kappa.B inhibitors and IKKs in normal prostate epithelial cells and prostate carcinoma (PC) cell lines LNCaP, MDA PCa 2b, DU145, PC3, and JCA1. The authors found that NF-.kappa.B was constitutively activated in human androgen-independent PC cell lines DU145, PC3, JCA1 as well as androgen-independent CL2 cells derived from LNCaP. In spite of a strong difference in constitutive .kappa.B binding, Western blot anal. did not reveal any significant variance in the expression of p50, p65, I.kappa.Bs, IKK.beta., and IKK.alpha. between primary prostate cells, androgen-dependent and androgen-independent PC cells. However, the authors found that in androgen-independent PC cells I.kappa.B.alpha. was heavily phosphorylated and displayed a faster turnover. Using an in vitro kinase assay the authors demonstrated constitutive activation of IKK in androgen-independent PC cell lines. Blockage of NF-.kappa.B activity in PC cells by dominant-neg. I.kappa.B.alpha. resulted in increased constitutive and TNF.alpha.-induced apoptosis. These data suggest that increased IKK activation leads to the constitutive activation of NF-.kappa.B "survival signaling" pathway in androgen-independent PC cells. This may be important for the support of their androgen-independent status and growth advantage.

L73 ANSWER 42 OF 136 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 29
AB A method is provided for identifying, evaluating, or making a compd. or agent, e.g., a candidate compd. or agent, for treatment of a disorder characterized by insulin resistance. The method includes evaluating the ability of a compd. or agent to interact with, e.g. **bind**, **IKK**-.beta., to thereby identify a compd. or agent for the treatment of a disorder characterized by insulin resistance. The invention also features compds. for treating insulin resistance identified by such methods, and methods of treating a subject having a disorder characterized by insulin resistance by administering such agents.

L73 ANSWER 54 OF 136 MEDLINE DUPLICATE 31
AB The IkappaB kinase (IKK) complex, composed of two catalytic subunits (IKKalpha and IKKbeta) and a regulatory subunit (IKKgamma), is the key enzyme in activation of nuclear factor kappaB (NF-kappaB). To study the mechanism and structure of the complex, we wanted to recombinantly express IKK in a model organism that lacks IKK. For this purpose, we have recombinantly reconstituted all three subunits together in yeast and have found that it is biochemically similar to IKK isolated from human cells. We show that there is one regulatory subunit per kinase subunit. Thus, the core subunit composition of IKKalpha.beta.gamma complex is alpha(1)beta(1)gamma(2), and the core subunit composition of IKKbeta.gamma is beta(2)gamma(2). The activity of the IKK complex (alpha+beta+gamma or beta+gamma) expressed in yeast (which lack NF-kappaB and IKK) is 4-5-fold higher than an equivalent amount of IKK from nonstimulated HeLa cells. In the absence of IKKgamma, IKKbeta shows a level of activity similar to that of IKK from nonstimulated HeLa cells. Thus, IKKgamma activates IKK complex in the absence of upstream stimuli. Deleting the gamma **binding** domain of **IKKbeta** or **IKKalpha** prevented IKKgamma induced activation of IKK complex in yeast, but it did not prevent the incorporation of IKKgamma into IKK and large complex formation. The possibility of IKK complex being under negative control in mammalian cells is discussed.

L73 ANSWER 68 OF 136 SCISEARCH COPYRIGHT 2003 THOMSON ISI

L73 ANSWER 79 OF 136 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

L73 ANSWER 93 OF 136 MEDLINE DUPLICATE 58
AB The adapter protein RIP plays a crucial role in NF-kappaB activation by TNF. Here we show that triggering of the p55 TNF receptor induces **binding** of RIP to NEMO (**IKKgamma**), a component of the I-kappa-B-kinase (IKK) "signalosome" complex, as well as recruitment of RIP to the receptor together with the three major signalosome components, NEMO, IKK1 and IKK2, and some kind of covalent modification of the recruited RIP molecules. It also induces binding of NEMO to the signaling inhibitor A20, and recruitment of A20 to the receptor. Enforced expression of NEMO in cells revealed that NEMO can both promote and block NF-kappaB activation and dramatically augments the phosphorylation of c-Jun. The findings suggest that the signaling activities of the **IKK** signalosome are regulated through **binding** of NEMO to RIP and A20 within the p55 TNF receptor complex.

L73 ANSWER 99 OF 136 HCAPLUS COPYRIGHT 2003 ACS
AB The cDNA encoding a novel low-mol.-wt. G protein-activating **Spa-1** protein is isolated from human peripheral lymphocytes treated with PHA and its amino acid sequence deduced. Expression of the cDNA in transgenic insect cells is demonstrated. Gene **Spa-1** is mapped to human chromosome 11q13.3. Methods of recombinant prepn. of **Spa-1** protein are also claimed.

L73 ANSWER 105 OF 136 MEDLINE DUPLICATE 66
AB The activation of NF-kappaB by receptors in the tumor necrosis factor (TNF) receptor and Toll/interleukin-1 (IL-1) receptor families requires the TRAF family of adaptor proteins. Receptor oligomerization causes the recruitment of TRAFs to the receptor complex, followed by the activation of a kinase cascade that results in the phosphorylation of IkappaB. TANK is a TRAF-binding protein that can inhibit the binding of TRAFs to receptor tails and can also inhibit NF-kappaB activation by these receptors. However, TANK also displays the ability to stimulate TRAF-mediated NF-kappaB activation. In this report, we investigate the mechanism of the stimulatory activity of TANK. We find that TANK interacts with TBK1 (TANK-**binding** kinase 1), a novel **IKK** -related kinase that can activate NF-kappaB in a kinase-dependent manner. TBK1, TANK and TRAF2 can form a ternary complex, and complex formation appears to be required for TBK1 activity. Kinase-inactive TBK1 inhibits TANK-mediated NF-kappaB activation but does not block the activation mediated by TNF-alpha, IL-1 or CD40. The TBK1-TANK-TRAF2 signaling complex functions upstream of NIK and the IKK complex and represents an alternative to the receptor signaling complex for TRAF-mediated activation of NF-kappaB.

=> save temp l73 ikkispa1/a
ANSWER SET L73 HAS BEEN SAVED AS 'IKKISPA1/A'

=> log y

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FULL ESTIMATED COST	188.79	189.00

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2	L2	538092	complex	USPAT; US-PGPUB	2003/05/09 09:04
3	L3	83	1 same 2	USPAT; US-PGPUB	2003/05/09 15:01
4	L4	17	spa-1	USPAT; US-PGPUB	2003/05/09 14:59
5	L5	2	1 and 4	USPAT; US-PGPUB	2003/05/09 14:59
6	L6	4	4 same 2	USPAT; US-PGPUB	2003/05/09 15:01

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1	L1	323	ikk$2	USPAT; US-PGPUB	2003/05/09 09:04
2	L2	538092	complex	USPAT; US-PGPUB	2003/05/09 09:04
3	L3	83	1 same 2	USPAT; US-PGPUB	2003/05/09 09:05

PGPUB-DOCUMENT-NUMBER: 20030087856

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030087856 A1

TITLE: Antisense modulation of IL-1 receptor-associated kinase-4 expression

PUBLICATION-DATE: May 8, 2003

INVENTOR-INFORMATION:

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Freier, Susan M.	San Diego	CA	US	

APPL-NO: 09/ 966451

DATE FILED: September 28, 2001

US-CL-CURRENT: 514/44, 435/375 , 514/81 , 536/23.2

ABSTRACT:

Antisense compounds, compositions and methods are provided for modulating the expression of IL-1 receptor-associated kinase-4. The compositions comprise antisense compounds, particularly antisense oligonucleotides, targeted to nucleic acids encoding IL-1 receptor-associated kinase-4. Methods of using these compounds for modulation of IL-1 receptor-associated kinase-4 expression and for treatment of diseases associated with expression of IL-1 receptor-associated kinase-4 are provided.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (7):

[0005] The IL-1 signaling pathway in mammals is analogous to the Toll pathway in Drosophila melanogaster. Homologues of the IL-1 receptor-associated kinases are found in D. melanogaster (Pelle) and in plants (Pto), and in these systems, the kinases have been shown to be components of a signal transduction system leading to the activation of NF-.kappa.B. A model for the signaling pathway in which IL-1 receptor associated kinase-4 is likely to function is as follows: when cells receive the extracellular IL-1 signal, a **complex** between IL-1RI and IL-1RAcP is formed (Huang et al., Proc. Natl. Acad. Sci. U.S.A., 1997, 94, 12829-12832), the cytosolic adapter protein MyD88 interacts with IL-1RAcP in the receptor **complex** (Burns et al., J. Biol. Chem., 1998, 273, 12203-12209), and MyD88 rapidly recruits a IL-1 receptor-associated kinase into the **complex**. Tollip also interacts with IL-1RAcP and is believed to block autophosphorylation of the IL-1 receptor-associated kinase or its association

with another kinase; thus, the association of Tollip with the IL-1 receptor-associated kinase is inhibitory (Burns et al., Nat. Cell Biol., 2000, 2, 346-351). At some point after its IL-1-dependent association with the receptor **complex,** the IL-1 receptor-associated kinase may be extensively phosphorylated and its own serine/threonine kinase catalytic activity activated (Cao et al., Science, 1996, 271, 1128-1131). IL-1 receptor-associated kinase-1 is known to interact with an adapter protein, TRAF6, a protein critical for IL-1-dependent activation of NF-.kappa.B, which then dissociates from the receptor **complex**. TRAF6 relays a signal via NF-.kappa.B-inducing kinase (NIK) to two I-.kappa.B kinases (**IKK-1** and -2), culminating in activation of NF-.kappa.B (Bacher et al., FEBS Lett., 2001, 497, 153-158; Jefferies et al., Mol. Cell. Biol., 2001, 21, 4544-4552; O'Neill and Greene, J. Leukoc. Biol., 1998, 63, 650-657).

PGPUB-DOCUMENT-NUMBER: 20030087411

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030087411 A1

TITLE: Death associated kinase containing ankyr in repeats (DAKAR) and methods of use

PUBLICATION-DATE: May 8, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 164080

DATE FILED: June 4, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60295959 20010604 US

non-provisional-of-provisional 60334362 20011129 US

US-CL-CURRENT: 435/194, 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

This invention relates to DAKAR, a new member of the serine/threonine kinase family, methods of making such polypeptides, and to methods of using them to treat conditions associated with apoptosis and epithelial proliferation and differentiation, as well as methods to identify compounds that alter DAKAR-associated cellular activities.

PRIORITY OF THE APPLICATION

[0001] This application claims the benefit of priority to U.S. Provisional Application No. 60/295,959, filed Jun. 4, 2001, and U.S. Provisional Application No. 60/334,362, filed Nov. 29, 2001.

---------- KWIC ---------

Detail Description Paragraph - DETX (50):

[0078] DAKAR may mediate its cellular effects in cells by interacting with a

number of substrates and cellular pathways. These may either be substrates that become phosphorylated, or possibly binding partners for the ankyrin repeats. In one embodiment of the present invention, a possible phosphorylation candidate is IkB and its associated subunits. In one particular embodiment, DAKAR functions to phosphorylate or form a **complex** with IKK.alpha., and may function in a pathway that signals to NFkB specifically in response to developmental or differentiation cues. In alternative embodiments, this pathway does not involve inflammatory signals. These embodiments would be consistent with the observed phenotypes of IKK.alpha..sup.-/- and DAKAR.sup.-/- embryos, and the signaling properties reported for IKK.alpha..sup.-/- cells. Existence of such a pathway might be analogous to reports for the **IKK** kinase NIK. Studies from NIK.sup.-/- cells indicate that NIK does not participate in **IKK** activation in response to either TNF or IL-1, but seems to specifically mediate signals in response to lymphotoxin-.alpha. (LT.alpha.). Moreover, yeast two hybrid and protein interaction studies show that NIK strongly and preferentially interacts with IKK.alpha.. In alternative embodiments, IKK.alpha. as well as other components of the **IKK complex** (IKK.beta. and IKK.gamma.) may be implicated in their ability to associate with or be phosphorylated by DAKAR. Similarly, components downstream in the signaling pathway such as the IkBs may also be implicated in DAKAR associated cellular events. For example, the ankyrin repeats of IkB are stacked helical domains which bind to the Re1 homology region (RHR) of NFkB, thereby masking its NLS. DAKAR may also bind NFkB directly in a similar manner, or perhaps to other ankyrin repeat-containing IkB proteins. DAKAR contains a putative nuclear localization site (NLS) at amino acids 469 to 482 of the polypeptide depicted in SEQ ID NO:7, and therefore is likely to undergo subcellular localization.

Detail Description Paragraph - DETX (97):

[0125] In another embodiment, a screening assay may comprise (a) culturing wild-type and/or DAKAR.sup.-/- cells, cell lysates or subcellular fractions in the presence or absence of an active compound that may enhance or inhibit directly or indirectly DAKAR-mediated association with, phosphorylation of and/or degradation of IkB, as well as subunits of the **IKK complex** including IKK.alpha., IKK.beta. and IKK.gamma., and (b) assessing the relative difference in measurable DAKAR-mediated association, phosphorylation and/or degradation of IkB by conventional techniques, such as IkB and IKK.alpha., IKK.beta. and IKK.gamma.-specific probes.

Detail Description Paragraph - DETX (142):

[0170] In other aspects of the present invention, DAKAR polypeptides, polynucleotides, fragments, variants, muteins, fusion proteins, antagonists, agonists, antibodies, and binding partners etc. may be used to enhance or inhibit apoptosis; DAKAR-associated NF.kappa.B activation and/or nuclear localization of NF.kappa.B, as well as NF.kappa.B subunits, such as the p65 subunit; DAKAR-mediated phosphorylation of one or more substrates, as well as association with binding domains such as catalytic domains of other proteins, which may include for example any of the calcium-dependent protein kinases, such as any of the protein kinase C (PKC) isoforms (PKC.delta. and/or PKC.beta., and the like); DAKAR-mediated association with, phosphorylation of

and/or degradation of IkB, as well as subunits of the **IKK complex** including IKK.alpha., IKK.beta. and IKK.gamma., DAKAR-mediated modulation of transcription and/or translation of differentiation markers, such as, but not limited to filaggrin, profilaggrin, involucrin, keratin markers (such as K1, K2, K2e, K2p, K4, K5, K6, K8, K9, K10, K13, K14, K16, K17, K18, K19, and the like); DAKAR-mediated cellular proliferation; DAKAR-associated caspase activity, modulate regulators of apoptosis and epidermal differentiation, such as members of the Bc1-2 family, including, but not limited to Bc1-2, Bc1-x, bax and bak; mediate intracellular signals in response to Lymphtoxin-.alpha.; modulate activity of c-Myc; and to modulate cell cycle regulators, such as cyclin-dependent kinase inhibitors, including for example p16, p21 and p27, as well as positive regulators such as cyclin A, cdk2 and/or cdc2; and thereby alleviate symptoms of diseases and disorders related thereto.

PGPUB-DOCUMENT-NUMBER: 20030084471

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030084471 A1

TITLE: Methods and compositions for RNA interference

PUBLICATION-DATE: May 1, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 055797

DATE FILED: January 22, 2002

RELATED-US-APPL-DATA:

child 10055797 A1 20020122

parent continuation-in-part-of PCT/US01/08435 20010316 US PENDING

non-provisional-of-provisional 60189739 20000316 US

non-provisional-of-provisional 60243097 20001024 US

US-CL-CURRENT: 800/278, 435/455

ABSTRACT:

The present invention provides methods for attenuating gene expression in a cell using gene-targeted double stranded RNA (dsRNA). The dsRNA contains a nucleotide sequence that hybridizes under physiologic conditions of the cell to the nucleotide sequence of at least a portion of the gene to be inhibited (the "target" gene).

RELATED APPLICATIONS

[0001] This application is a continuation-in-part of PCT application PCT/US01/08435, filed Mar. 16, 2001, and claims the benefit of U.S. Provisional applications U.S. SNo. 60/189,739 filed Mar. 16, 2000 and U.S. SNo. 60/243,097 filed Oct. 24, 2000. The specifications of such applications are incorporated by reference herein.

---------- KWIC ---------

Detail Description Paragraph - DETX (11):

[0106] In certain embodiments, the cells can be treated with an agent(s) that inhibits the general double-stranded RNA response(s) by the host cells, such as may give rise to sequence-independent apoptosis. For instance, the cells can be treated with agents that inhibit the dsRNA-dependent protein kinase known as PKR (protein kinase RNA-activated). Double stranded RNAs in mammalian cells typically activate protein kinase PKR and lead to apoptosis. The mechanism of action of PKR includes phosphorylation and inactivation of eIF2.alpha. (Fire (1999) Trends Genet 15: 358). It has also been reported that induction of NF-.kappa.B by PKR is involved in apoptosis commitment and this process is mediated through activation of the **IKK complex**. This sequence-independent response may reflect a form of primitive immune response, since the presence of dsRNA is a common feature of many viral lifecycles.

PGPUB-DOCUMENT-NUMBER: 20030082638

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030082638 A1

TITLE: Methods for screening osteogenic compounds

PUBLICATION-DATE: May 1, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 272194

DATE FILED: October 15, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60329231 20011012 US

non-provisional-of-provisional 60329393 20011015 US

non-provisional-of-provisional 60329360 20011015 US

non-provisional-of-provisional 60328876 20011012 US

US-CL-CURRENT: 435/7.2, 514/2

ABSTRACT:

Method of screening osteogenic compounds utilizing RANK and/or RANK pathway, compositions comprising compounds selected thereby and method for enhancing bone mass comprising administering to a subject an effective amount of said compositions.

[0001] This application is related to and claims the benefit of the following U.S. applications, which are incorporated herein by reference as if restated here in full: Serial No. 60/277,855 filed Mar. 22, 2001; Ser. No. 10/105,057 filed Mar. 22, 2002; Serial No. 60/311,163 filed Aug. 9, 2001; Ser. No. 10/215,446 filed Aug. 9, 2002; Serial No. 60/329,231 filed Oct. 12, 2001; Serial No. 60/329,393 filed Oct. 15, 2001; Serial No. 60/329,360 filed Oct. 15, 2001; Serial No. 60/328,876 filed Oct. 12, 2001; U.S. non-provisional entitled RANKL Mimics and Uses Thereof, LAM, et al., filed Oct. 15, 2002; U.S. non-provisional entitled Bone-Anti Resorptive Compounds, LAM, et al., filed Oct. 15, 2002.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (19):

[0018] In a preferred embodiment, the proteins that are assayed include proto-oncogene proteins, including c-Fos, and intracellular kinases, including ERK1/2, JNK, p38, PI3 kinase, Akt, and **IKK**. **IKK** functions as an upstream regulator of the transcriptional **complex** comprising the NFKB family. These NFKB members are well know to those versed in the art. Activation of the NFKB pathway is assessed by performing electrophoretic mobility shift assays (EMSA) on nuclear extracts. More preferably the assayed kinases are MAP kinases, including ERK1/2, JNK, and p38. Most preferably, the assayed kinase is ERK1/2.

PGPUB-DOCUMENT-NUMBER: 20030082511

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030082511 A1

TITLE: Identification of modulatory molecules using inducible promoters

PUBLICATION-DATE: May 1, 2003

INVENTOR-INFORMATION:

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Dunnington, Damien J.	San Diego	CA	US	
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APPL-NO: 09/ 965201

DATE FILED: September 25, 2001

US-CL-CURRENT: 435/4, 435/6

ABSTRACT:

Methods for identifying an ion channel modulator, a target membrane receptor modulator molecule, and other modulatory molecules are disclosed, as well as cells and vectors for use in those methods. A polynucleotide encoding target is provided in a cell under control of an inducible promoter, and candidate modulatory molecules are contacted with the cell after induction of the promoter to ascertain whether a change in a measurable physiological parameter occurs as a result of the candidate modulatory molecule.

---------- KWIC ---------

Detail Description Table CWU - DETL (13):

(soluble) HMGCS2 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial) HMOX1 Hs.75967; heme oxygenase (decycling) 1 HMOX2 Hs.83853; HO-2; heme oxygenase (decycling) 2 HNK-1ST HNK-1 sulfotransferase HNMT histamine N-methyltransferase HPD Hs.89831; PPD; 4-hydroxyphenylpyruvate dioxygenase HPGD hydroxyprostaglandin dehydrogenase 15-(NAD) HPN "hepsin (transmembrane protease, serine 1); Hs.823; TMPRSS1; hepsin" HPRT1 Hs.82314; HPRT: HGPRT; hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome) HPRT2 hypoxanthine phosphoribosyltransferas- e 2 HPRTP1 hypoxanthine phosphoribosyltransferase pseudogene 1 HPRTP2 hypoxanthine phosphoribosyltransferase pseudogene 2 HPRTP3 hypoxanthine phosphoribosyltransferase pseudogene 3 HPRTP4 hypoxanthine phosphoribosyltransferase pseudogene 4 HPSE HPA; HSE1; heparanase HRMT1LI

"HMT1 (hnRNP methyltransferase, S. cerevisiae)-like 1; PRMT2" HRMT1L2 "HMT1 (hnRNP methyltransferase, S. cerevisiae)-like 2; HCP1; PRMT1" HS3ST1 heparan sulfate (glucosamine) 3-O-sulfotransferase 1 HS3ST2 heparan sulfate (glucosamine) 3-O-sulfotransferase 2 HS3ST3A1 heparan sulfate (glucosamine) 3-O-sulfotransferase 3A1 HS3ST3A2 heparan sulfate (glucosamine) 3-O-sulfotransferase 3A2 HS3ST3B1 heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1 HS3ST3B2 heparan sulfate (glucosamine) 3-O-sulfotransferase 3B2 HS3ST4 heparan sulfate (glucosamine) 3-O-sulfotransferase 4 HS6ST heparan-sulfate 6-sulfotransferase HSA9947 putative ATPase HSCR2 HSCR; Hirschsprung disease 2 HSD11B1 HSD11; HSD11B; hydroxysteroid (11-beta) dehydrogenase 1 HSD11B2 hydroxysteroid (11-beta) dehydrogenase 2 HSD17B1 HSD17; EDHB17; EDH17B2; hydroxysteroid (17-beta) dehydrogenase 1 HSD17B2 Hs.181; hydroxysteroid (17-beta) dehydrogenase 2 HSD17B3 Hs.477; hydroxysteroid (17-beta) dehydrogenase 3 HSD17B4 hydroxysteroid (17-beta) dehydrogenase 4 HSD17B5 hydroxysteroid (17-beta) dehydrogenase 5 HSD17BP1 HSD17; EDHB17; EDH17B1; hydroxysteroid (17-beta) dehydrogenase pseudogene 1 HSD3B1 "Hs.38586; HSDB3; HSD3B; hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1" HSD3B2 "hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2" HSD3B3 "HSD3B3-LSB; hydroxy-delta-5-steroid dehydrogenase, 3 beta- C(27); giant cell hepatitis, neonatal" HSD3BP1 "hydroxy-delta-5-steroid dehydrogenase, 3 beta, pseudogene 1" HSD3BP2 "hydroxy-delta-5-steroid dehydrogenase, 3 beta, pseudogene 2" HSD3BP3 "hydroxy-delta-5-steroid dehydrogenase, 3 beta, pseudogene 3" HSD3BP4 "hydroxy-delta-5-steroid dehydrogenase, 3 beta, pseudogene 4" HSD3BP5 "hydroxy-delta-5-steroid dehydrogenase, 3 beta, pseudogene 5" HTOR 5-hydroxytryptamine (serotonin) oxygenase regulator HTR7 Hs.73739; 5-hydroxytryptamine (serotonin) receptor 7 (adenylate cyclase-coupled) HU-K5 lysophospholipase-like HYAL1 hyaluronoglucosaminidase 1; LUCA1; HYAL-1 HYAL2 LUCA-2; hyaluronoglucosaminidase 2 HYAL3 hyaluronoglucosaminidase 3; LUCA-3; LUCA 14; Minna14 HYL HYL-PEN; hematopoietic consensus tyrosine-lacking kinase IARS Hs.89412; ILRS; isoleucine-tRNA synthetase; Hs.78770 IBD1 inflammatory bowel disease 1; Crohn disease IBGC1 idiopathic basal ganglia calcification 1; BGCI; IBGC; Fahr disease ICB-1 basement membrane-induced gene IDH1 "isocitrate dehydrogenase 1 (NADP+), soluble" IDH2 "Hs.105969; isocitrate dehydrogenase 2 (NADP+), mitochondrial" IDH3A isocitrate dehydrogenase 3 (NAD+) alpha IDH3B isocitrate dehydrogenase 3 (NAD+) beta IDH3G isocitrate dehydrogenase 3 (NAD+) gamma IDI1 isopentenyl diphosphate delta isomerase IDO "Hs.840; indole 2,3-dioxygenase" IDS iduronate 2-sulfatase (Hunter syndrome); Hs.79285; SIDS IDSP1 IDS2; iduronate 2-sulfatase pseudogene 1 IDUA "iduronidase, alpha-L-; Hs.89560" IKBKAP "inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase **complex**-associated protein; IKAP" IKBKB "inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta; **IKK2**; NFKBIKB; **IKK**-beta" IKBKG "inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma; NEMO; **IKK**-gamma" IL17 CTLA8; interleukin 17 (cytotoxic T-lymphocyte-associated serine esterase 8); Hs.41724 ILF3 "interleukin enhancer binding factor 3, 90 kD; M-phase phosphoprotein 4; NF90; MPP4; DRBP76; NFAR-1; MPHOSPH4; MMP4" ILK integrin-linked kinase; Hs.6196 ILVBL AHAS; ILV2H; ilvB (bacterial acetolactate synthase)-like IMPA1 IMPA; inositol(myo)-1 (or 4)-monophosphatase 1 IMPA2 inositol(myo)-1 (or 4)-monophosphatase 2 IMPDH1 Hs.850; IMP (inosine monophosphate) dehydrogenase 1; sWSS2608 IMPDH2 Hs.75432; IMP (inosine monophosphate) dehydrogenase 2 IMPDHL1 IMP (inosine

monophosphate) dehydrogenase-like 1 INDO "IDO; indoleamine-pyrrole 2,3 dioxygenase" INMT ndolethylamine N-methyltransferase; thioester 5-methyltransferase-like; indolethylamine N-methyltransferase INPP1 inositol polyphosphate-1-phosphatase; Hs.32309 INPP3 inositol polyphosphate-3-phosphatase INPP4A INPP4; inositol polyphosphate-4-phosphatase INPP4B "inositol polyphosphate-4-phosphatase, type II, 105 kD" INPP5A "inositol trisphosphate-5-phosphatase, 40 kD; inositol polyphosphate-5- phosphatase, 40 kD" INPP5B "inositol polyphosphate-5-phosphatase, 75 kD" INPP5C "inositol polyphosphate-5-phosphatase, 120 kD" INPP5D "inositol polyphosphate-5-phosphatase, 145 kD; SHIP; hp51CN" INPPL1 Hs.75339; inositol polyphosphate phosphatase-like 1; SHIP2 IQGAP2 IQ motif containing GTPase activating protein 2 IRAK-M interleukin-1 receptor-associated kinase M IRAK1 interleukin-1 receptor-associated kinase; IRAK; Pelle (Drosophila) homolog; pelle IRAK2 interleukin-1 receptor-associated kinase 2; IRAK-2 ITK IL2-inducible T-cell kinase; EMT; T-cell-specific tyrosine kinase; homolog of mouse T-cell itk/tsk tyrosine kinase; PSCTK2 ITPA inosine triphosphatase (nucleoside triphosphate pyrophosphatase) ITPK1 "inositol 1,3,4-trisphosphate 5/6 kinase" ITPKA "Hs.2722; inositol 1,4,5-trisphosphate 3-kinase A" ITPKB "Hs.78877; inositol 1,4,5-trisphosphate 3-kinase B" IVD Hs.77510; isovaleryl Coenzyme A dehydrogenase JAK1 JAK1A; Janus kinase 1 (a protein tyrosine kinase) JAK2 Janus kinase 2 (a protein tyrosine kinase) JAK3 "Hs.99877; L-JAK; Janus kinase 3 (a protein tyrosine kinase, leukocyte)" JTK5A JTK5A protein tyrosine kinase JTK5B JTK5B protein tyrosine kinase KAPPA "Kappa transcript, coding region similar to kinases" KAR Aromatic alpha-keto acid reductase KARS lysyl-tRNA synthetase KATII kynurenine aminotransferase II KATNA1 katanin p60 (ATPase-containing) subunit A1 KDR kinase insert domain receptor (a type III receptor tyrosine kinase); Hs.12337; FLK1; VEGFR2 KHK ketohexokinase (fructokinase); Hs.81454 KIAA0566 "ATP#; ATPase type IV, phospholipid transporting (P-type) (putative)" KIAA0611 "ATP#; ATPase type IV, phospholipid-transporting (P-type), (putative)" KIAA0660 G3BP2; Ras-GTPase activating protein SH3 domain-binding protein 2 KIAA0901 HDAC6; histone deacetylase 6 KIAA0928 helicase-moi KIP2 DNA-dependent protein kinase catalytic subunit-interacting protein 2 KLK6 "PRSS9; kallikrein 6 (neurosin, zyme); protease, serine, 9 (neurosin); protease M" KLK7 "PRSS6; kallikrein 7 (chymotryptic, stratum corneum); SCCE; protease, serine, 6 (chymotryptic, stratum corneum)" KMO kynurenine 3-monooxygenase (kynurenine 3-hydroxylase) KNPEP lysyl aminopeptidase (aminopeptidase Co) KSR KSR1; kinase suppressor of ras KWE keratolytic winter erythema (Oudtshorn skin disease) KYN KYN-PEN; kynureninase KYNU kynureninase (L-kynurenine hydrolase) LAP70 "apyrase lysosomal" LARGE like-glycosyltransferase; KIAA0609 LARS leucyl-tRNA synthetase LAS lipoic acid synthetase LCAT Hs.23513; lecithin-cholesterol acyltransferase; Norum disease; fish-eye disease LCB2 "KIAA0526; serine palmitoyltransferase, subunit II" LCK Hs.1765; lymphocyte-specific protein tyrosine kinase LCT Hs.2251; lactase LDHA Hs.2795; lactate dehydrogenase A LDHAL1 lactate dehydrogenase A-like 1 LDHAL2 lactate dehydrogenase A-like 2 LDHAL3 lactate dehydrogenase A-like 3 LDHAL4 lactate dehydrogenase A-like 4 LDHAL5 lactate dehydrogenase A-like 5 LDHB Hs.74545; lactate dehydrogenase B LDHBL1 lactate dehydrogenase B-like 1 LDHBP LDHBL2; lactate dehydrogenase B pseudogene LDHC Hs.99881; lactate dehydrogenase C; Hs.511

PGPUB-DOCUMENT-NUMBER: 20030077683

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030077683 A1

TITLE: IKK-alpha proteins, nucleic acids and methods

PUBLICATION-DATE: April 24, 2003

INVENTOR-INFORMATION:

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Regnier, Catherine	South San Francisco	CA	US	

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DATE FILED: September 13, 2002

RELATED-US-APPL-DATA:

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parent-patent 6479266 US

child 09109986 19980702 US

parent continuation-of 08890854 19970710 US GRANTED

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child 08890854 19970710 US

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ABSTRACT:

The invention provides methods and compositions relating to an I.kappa.B kinase, IKK-.alpha., and related nucleic acids. The polypeptides may be produced recombinantly from transformed host cells from the disclosed IKK-.alpha. encoding nucleic acids or purified from human cells. The invention provides isolated IKK-.alpha. hybridization probes and primers capable of specifically hybridizing with the disclosed IKK-.alpha. genes, IKK-.alpha.-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35USC120 to U.S. Ser. No. 09/109,986, filed on Jul. 2, 1998, now allowed, which claims priority to U.S. Ser. No. 08/890,854, filed Jul. 10, 1997, now U.S. Pat. No. 6,235,512, which claims priority to U.S. Ser. No. 08/887,115 filed Jul. 1, 1997, abandoned, all of which are incorporated herein by reference.

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Summary of Invention Paragraph - BSTX (8):

[0008] Here, we disclose a novel human kinase I.kappa.B Kinase, **IKK**-.alpha., as a NIK-interacting protein. **IKK**-.alpha. has sequence similarity to the conceptual translate of a previously identified open reading frame (SEQ ID NO:5) postulated to encode a serine-threonine kinase of unknown function (`Conserved Helix-loop-helix Ubiquitous Kinase` or CHUK, Connelly and Marcu, 1995; Mock et al., 1995). Catalytically inactive mutants of TKK-.alpha. are shown to suppress NF-.kappa.B activation induced by TNF and IL-1 stimulation as well as by TRAF and NIK overexpression; transiently expressed **IKK**-.alpha. is shown to associate with the endogenous I.kappa.B.alpha. **complex; and IKK**-.alpha. is shown to phosphorylate I.kappa.B.alpha. on serines 32 and 36.

Summary of Invention Paragraph - BSTX (16):

[0014] The claimed **IKK**-.alpha. polypeptides are isolated or pure: an "isolated" polypeptide is unaccompanied by at least some of the material with which it is associated in its natural state, preferably constituting at least about 0.5%, and more preferably at least about 5% by weight of the total polypeptide in a given sample and a pure polypeptide constitutes at least about 90%, and preferably at least about 99% by weight of the total polypeptide in a given sample. In a particular embodiments, **IKK**-.alpha. polypeptides are isolated from a MKP-1 precipitable **complex,** isolated from a **IKK complex,** and/or isolated from **IKK**-.beta.. The **IKK**-.alpha. polypeptides and polypeptide domains may be synthesized, produced by recombinant technology, or purified from mammalian, preferably human cells. A wide variety of molecular and biochemical methods are available for biochemical synthesis, molecular expression and purification of the subject compositions, see e.g. Molecular Cloning, A Laboratory Manual (Sambrook, et al Cold Spring Harbor Laboratory), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY) or that are otherwise known in the art.

Summary of Invention Paragraph - BSTX (58):

[0055] The interaction of **IKK**-.alpha. with NIK was confirmed in mammalian cell coimmunoprecipitation assays. Human **IKK**-.alpha. containing an N-terminal Flag epitope tag was transiently coexpressed in 293 human embryonic kidney

cells with Myc epitope-tagged NIK or HA epitope-tagged TRAF proteins. Cell lysates were immunoprecipitated using a monoclonal antibody against the Flag epitope, and coprecipitating NIK or TRAF proteins were detected by immunoblot analysis with an anti-Myc or anti-HA monoclonal antibodies. In this assay, **IKK**-.alpha. was able to coprecipitate NIK confirming the interaction between both proteins as detected for **IKK**-.alpha. by yeast two-hybrid analysis. Also, a deletion mutant **IKK**-.alpha. protein lacking most of the N-terminal kinase domain (**IKK**-.alpha..sub.(307-745)) was able to associate with NIK, indicating that the .alpha.-helical C-terminal half of **IKK**-.alpha. mediates the interaction with NIK. In contrast to NIK, **IKK**-.alpha. failed to associate with either TRAF2 or TRAF3. However, when NIK was coexpressed with **IKK**-.alpha. and TRAF2, strong coprecipitation of TRAF2 with **IKK**-.alpha.. was detected, indicating the formation of a ternary **complex** between **IKK**-.alpha., NIK and TRAF2.

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TITLE: Biologically active alternative form of the IKKalpha IkappaB kinase

PUBLICATION-DATE: April 17, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 188937

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US-CL-CURRENT: 435/6, 435/194 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

The present invention provides isolated I.kappa.B kinases that regulate NF.kappa.B gene transcription that lack both a leucine zipper like .alpha.-helix domain and helix-loop-helix domain. Also provided are the amino acid sequences of these kinases and the nucleotide sequence encoding these kinases, and other related protein and nucleic acid molecules.

---------- KWIC ---------

Detail Description Paragraph - DETX (6):

[0039] Akin to IKK.alpha./CHUK, the IKK.alpha.-.DELTA.LH and IKK.alpha.-.DELTA.Cm proteins are TNF-.alpha. inducible, NF-.kappa.B activating I.kappa.B.alpha. kinases. By a combination of NF-.kappa.B element driven luciferase gene reporter assays, immune **complex** kinase assays and co-immunoprecipitations with other known components of the approximately 700-900 kD **IKK complex,** the IKK.alpha.-.DELTA.LH and IKK.alpha.-.DELTA.Cm proteins were found to behave in a similar fashion to full length

IKK.alpha./CHUK by several criteria. First, expression plasmid dose response curves reveal that each form of IKK.alpha./CHUK activates a comparable level of NF-.kappa.B luciferase activity even at their limiting dosages (FIG. 4B). Second, each form of IKK.alpha./CHUK correctly phosphorylates I.kappa.B.alpha. (on serines 32 and 36) in response to TNF.alpha. signaling (FIG. 5A). Third, IKK.alpha.-.DELTA.Cm activates NF-.kappa.B and phosphorylates I.kappa.B.alpha. with an enzymatic time course superimposable with full length IKK.alpha./CHUK. (FIG. 5B.) Fourth, like IKK.alpha./CHUK, IKK.alpha.-.DELTA.Cm's ability to activate NF-.kappa.B is not appreciably enhanced by co-expression with IKK.beta. and is inhibited by a kinase inactive, ATP binding domain mutant of IKK.alpha./CHUK. Therefore, these isoforms of IKK.alpha./CHUK, which lack the LZ and H-L-H domains, retain a number of functions of the full length IKK.alpha./CHUK. It is surprising that the carboxy-tail domain of the full length IKK.alpha./CHUK does not significantly contribute to the kinase's functional activity.

Detail Description Paragraph - DETX (93):

[0125] Immune **complex** kinase assays. HEK293 cells (2.5.times.10 cells in 10 cm plates) were transfected with 10 .mu.g of kinase expression plasmid by the calcium phosphate method and stimulated 24 h later in DMEM with appropriate agonist at 37.degree. C. for the times indicated. Cells were washed with ice cold PBS and lysed with Triton X-100 lysis buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 50 mM NaF, 5 mM EDTA, 40 mM .beta.-glycerophosphate, 200 .mu.M sodium orthovanadate, 10.sup.-4M phenylmethyl-sulfonyl fluoride, 1 mg/ml leupeptin, 1 .mu.M pepstatin A, 1% Triton X-100). Proteins from lysates (500 .mu.g) were incubated with specific anti-HA (12CA5) or V5 epitope (Invitrogen Inc.) antibodies preadsorbed to protein A-Sepharose coated beads for 2 h at 4.degree. C. Immune complexes were washed three times with Triton X-100 lysis buffer and twice with kinase assay buffer (20 mM HEPES, pH 7.4, 20 mM MgCl.sub.2, 1 mM dithiothreitol, 10 mM p-nitrophenylphosphate). **IKKA** activity was assayed by resuspending the final pellet in 40 .mu.l of kinase buffer containing 50 .mu.M of [.alpha.-.sup.32P] ATP (5000 c.p.m./pmol) (Amersham) and 0.25 mg/ml of GST-I.kappa.B.alpha.(1-62). The reaction was incubated for 10 min at 30.degree. C. and stopped with Laemmli sample buffer. Samples were resolved on SDS-PAGE (10%) and phosphorylation determined by exposure in a phosphorimager (Molecular Dynamics).

PGPUB-DOCUMENT-NUMBER: 20030073097

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TITLE: TRAF6-regulated IKK activators (TRIKA1 and TRIKA2) and their use as anti-inflammatory targets

PUBLICATION-DATE: April 17, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 076918

DATE FILED: October 11, 2001

US-CL-CURRENT: 435/6, 435/21 , 435/7.92

ABSTRACT:

Proteins in the **IKK** and JNK signaling pathways, such as NF.kappa.B, are involved in the regulation of inflammatory diseases. Through phosphorylation and polyubiquitination, I.kappa.B proteins which sequester NF.kappa.B in the cytoplasm, are degraded by the ubiquitin-proteasome pathway releasing NF.kappa.B to the nucleus where it is activated. The present invention provides methods utilizing the composition of proteins in the **IKK,** JNK and ubiquitin-proteasome pathways such as, TRAF6 or TRAF2 (E3-ubiquitin protein ligase), TRIKA1/Uev1A/Ubc13 **complex** (E2-ubiquitin conjugating enzyme), and TRIKA2/TAK1 (protein kinase), in screening for candidate modulators involved in activation of the **IKK** and JNK pathways. The application further provides methods of utilizing the candidate modulators as drug therapeutics against inflammatory and immune diseases.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

Proteins in the **IKK** and JNK signaling pathways, such as NF.kappa.B, are involved in the regulation of inflammatory diseases. Through phosphorylation and polyubiquitination, I.kappa.B proteins which sequester NF.kappa.B in the cytoplasm, are degraded by the ubiquitin-proteasome pathway releasing NF.kappa.B to the nucleus where it is activated. The present invention provides methods utilizing the composition of proteins in the **IKK,** JNK and ubiquitin-proteasome pathways such as, TRAF6 or TRAF2 (E3-ubiquitin protein ligase), TRIKA1/Uev1A/Ubc13 **complex** (E2-ubiquitin conjugating enzyme), and

TRIKA2/TAK1 (protein kinase), in screening for candidate modulators involved in activation of the **IKK** and JNK pathways. The application further provides methods of utilizing the candidate modulators as drug therapeutics against inflammatory and immune diseases.

Summary of Invention Paragraph - BSTX (7):

[0007] NF.kappa.B provides a paradigm for a transcription factor that is regulated primarily via nuclear translocation (Sen and Baltimore, 1986; Baeuerle and Baltimore, 1988). NF.kappa.B plays a critical role in regulating the expression of a number of genes including cytokines, chemokines and other mediators of inflammatory and immune responses. NF.kappa.B is a dimeric transcription factor composed of p50 (NF.kappa.B1) and p60 (Re1A) subunits (Hatada et al., 2000). In unstimulated cells NF.kappa.B is bound to one of three I.kappa.B inhibitory proteins (I.kappa.B.alpha., I.kappa.B.beta., or I.kappa.B.epsilon.) which is responsible for sequestering the NF.kappa.B/I.kappa.B **complex** in the cytoplasm. Many of the signals that lead to the activation of NF.kappa.B converge on **IKK** kinase, which consists of the catalytic subunits IKK.alpha., IKK.beta., and the regulatory subunit Nemo (also known as IKK.gamma.).

Summary of Invention Paragraph - BSTX (8):

[0008] Upon stimulation by agonists, such as tumor necrosis factor .alpha. (TNF.alpha.) and interleukin 1.beta. (IL-1.beta.), I.kappa.B kinase (**IKK) complex** is activated and subsequently rapidly phosphorylates I.kappa.B proteins. I.kappa.B kinase **complex** can also be independently activated by the mitogen-activated protein kinase kinase kinase family MAP3K; such as the extracellular signal-regulated kinase kinase kinase 1 MEKK1, a kinase of the JNK/SAPK pathway; and NF.kappa.B inducing kinase NIK.

Summary of Invention Paragraph - BSTX (13):

[0012] Thus, in accordance with the present invention there is provided a method of screening for modulators of **IKK** and JNK activation comprising (a) providing a Ubc13/Uev1A **complex** and TRAF6 or TRAF2; (b) contacting the **complex** and TRAF6 or TRAF2 with a candidate modulator substance in the presence of E1, a plurality of ubiquitin molecules and ATP; (c) determining the formation of free polyubiquitin chains, wherein a change in poly-ubiquitin formation in the presence of the candidate modulator, as compared with poly-ubiquitin formation in the absence of the candidate modulator, indicates that the candidate modulator is an modulator of **IKK** and JNK activation.

Summary of Invention Paragraph - BSTX (15):

[0014] In another embodiment, there is provided a method of screening for modulators of **IKK** and JNK activation comprising (a) providing a Ubc13/Uev1A **complex,** TRAF6 or TRAF2; (b) contacting TAB1/TAB2/TAK1 **complex,** Ubc13/Uev1A

complex, TRAF6 or TRAF2 with a candidate modulator substance in the presence of E1, a plurality of ubiquitin molecules and ATP; (c) determining the polyubiquitination of TRAF6 or TRAF2, wherein a change in the phosphorylation state of **IKK** or MKK in the presence of the candidate modulator, as compared with the phosphorylation state of **IKK** or MKK in the absence of the candidate modulator, indicates that the candidate modulator is an modulator of **IKK** and JNK activation.

Summary of Invention Paragraph - BSTX (21):

[0020] In still yet a further embodiment, there is provided a method of screening for modulators of **IKK** and JNK activation comprising (a) providing TAK1, TAB1, TAB2, TRAF6 and **IKK complex**; (b) contacting TAK1, TAB1, TAB2, TRAF6 and **IKK complex** with a candidate modulator substance in the presence of E1, Ubc13/Uev1A, a plurality of ubiquitin molecules and ATP; (c) determining the phosphorylation state of I.kappa.B and MKK, wherein a change in the phosphorylation state of I.kappa.B and MKK in the presence of the candidate modulator, as compared with the phosphorylation state of I.kappa.B and MKK in the absence of the candidate modulator, indicates that the candidate modulator is an modulator of **IKK** and JNK activation.

Detail Description Paragraph - DETX (5):

[0033] Thus, in one embodiment of the present invention, the present inventors exploit the discovery that TRAF6 and TRAF2 proteins function as a ubiquitin ligases. In another embodiment, the invention involves use of the ubiquitin conjugating enzymes Ubc13 and UevA1 as part of a functional **complex** (TRIKA1) in activation of the **IKK** pathway. In yet another embodiment, TRIKA2, which comprises TAK1, TAB1 and TAB2 (TRIKA2/TAK1 **complex**), is identified as a new class of protein kinases whose activity is regulated by ubiquitination, thereby identifying a novel regulatory mechanism that is exploited in the present invention. In other embodiments, the JNK pathway also is regulated by TRIKA1 and TRIKA2 proteins, demonstrating cross-talk between the signaling pathways that can be exploited.

Detail Description Paragraph - DETX (8):

[0036] NF.kappa.B plays a critical role in regulating the expression of a number of genes including cytokines, chemokines and other mediators of inflammatory and immune responses. NF.kappa.B/Re1 transcription factor family consist of five mammalian family members which bind DNA as homodimers or heterodimers. NF.kappa.B is a dimeric transcription factor composed of p50 (NF.kappa.B1) and p60 (Re1A) subunits (Hatada et al., 2000) and is primarily regulated via nuclear translocation. In unstimulated cells NF.kappa.B is bound to one of three I.kappa.B inhibitory proteins (I.kappa.B.alpha., I.kappa.B.beta., or I.kappa.B.epsilon.) which is responsible for sequestering the NF.kappa.B/I.kappa.B **complex** in the cytoplasm. I.kappa.Bs retain NF.kappa.B in the cytoplasm by masking its nuclear localization signal. Many of the signals that lead to the activation of NF.kappa.B converge on **IKK** kinase

which consists of the catalytic subunits IKKB.alpha., IKK.beta., and the regulatory subunit Nemo (also known as IKKB.gamma.).

Detail Description Paragraph - DETX (9):

[0037] Upon stimulation by agonists, such as tumor necrosis factor a (TNF.alpha.) and interleukin 1.beta. (IL-1.beta.), I.kappa.B kinase (**IKK) complex** is activated and subsequently rapidly phosphorylates I.kappa.B proteins. This phosphorylation event is specific in that it occurs only on serine residues; Ser32 and 36 on I.kappa.B.alpha. and Ser 19 and 23 on I.kappa.B.beta.. I.kappa.B kinase **complex** can also be independently activated by the mitogen-activated protein kinase kinase kinase family MAP3K; such as the extracellular signal-regulated kinase kinase kinase 1 MEKK1, a kinase of the JNK/SAPK pathway; and NF.kappa.B inducing kinase NIK. Phosphorylation of I.kappa.B proteins leads to polyubiquitination and subsequent degradation of these proteins followed by the release of NF.kappa.B. (Ghosh et al, 1998).

Detail Description Paragraph - DETX (38):

[0066] TAK1 is a kinase, which is an enzyme that transfers a phosphate group to a target molecule. In measuring the activity of TAK1, a variety of different components are required. In addition to TAK1, a reaction mixture contains E1, Ubc13/Uev1A. TRAF6, ubiquitin, TAK1/TAB1/TAB2 (TRIKA2), **IKK complex,** I.kappa.B.alpha. and ATP. In this reaction, I.kappa.B.alpha. is the target; it may be fused to a protein such as GST for isolation purposes. After incubation in the presence or absence of inhibitors, the reactions can be quenched by adding 10-20 mM EDTA.

Detail Description Paragraph - DETX (44):

[0072] Biological interactions, such as polyubiquitin formation, the ubiquitination of TRAF6 or TRAF2, or the phosphorylation of **IKK** and MKK by the kinase activity of TRIKA2/TAK1 **complex,** can be examined by immunodetection methods, as discussed briefly above. Some immunodetection methods include enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoradiometric assay, fluoroimmunoassay, chemiluminescent assay, bioluminescent assay, and Western blot to mention a few. The steps of various useful immunodetection methods have been described in the scientific literature (e.g., Doolittle & Ben-Zeev, 1999; Gulbis & Galand, 1993; and De Jager et al., 1993).

Detail Description Paragraph - DETX (137):

[0163] Plasmids and Proteins. Ub and various Ub mutants were expressed in a modified E. coli strain, BL21(DE3)pJY2, to prevent misincorporation of lysines (You et al., 1999). E1 was purified from calf thymus by covalent affinity chromatography on Ub-Sepharose. Expression plasmids for GST-Ubch5c and GST-Ubc13 were kindly provided by Dr. Alan Weissman (NCI). I.kappa.B.alpha. and mutants were in vitro translated in wheat germ extract in the presence of

.sup.35S-methionine (Chen et al., 1995). **IKK complex** was purified form HeLa cells or from calf thymus using ATP-Sepharose (Upstate Biotechnology Inc.; Spencer et al., 1999). MEKK1 protein was expressed in baculovirus-infected Sf9 insect cells and purified as described previously (Lee et al, 1997). cDNAs for TRAF6 and a truncated form of TRAF6 fused to gyrase B (T6RZC) were gifts from Dr. Jun Ichiro-Inoue (University of Tokyo). These cDNA were subcloned into pFast-Bac (Gibco-BRL) for expression in Sf9 cells as His.sub.6-tagged proteins. While both TRAF6 and T6RZC work indistinguishably in **IKK** activation in vitro, T6RZC consistently gave better expression and was used in place of TRAF6 in most experiments. Site-directed mutagenesis of the RING finger of TRAF6 at Cys-70 (C70A) and Cys-85/His-87 (C85A/H87A) was carried out using the QuikChange kit (Stratagene). NEMO cDNA was cloned from human placenta cDNA library by Polymerase Chain Reaction (PCR), and then subcloned into pFast-Bac for expression in Sf9 cells. All constructs were verified by automatic DNA sequencing. His.sub.6-tagged proteins were purified using nickel columns (Qiagen).

Detail Description Paragraph - DETX (139):

[0165] In vitro Assay for **IKK** Activation. To detect endogenous **IKK** activation, cell extracts (5 mg/ml) were incubated with TRAF6 (or, T6RZC, 0.1 .mu.M) together with an ATP regenerating buffer (Chen et al., 1995). After incubation at 30.degree. C. for 1 hour, the reaction was analyzed by immunoblotting with a phospho-I.kappa.B.alpha. specific antibody. To identify intermediary factors involved in TRAF6-mediated **IKK** activation, the inventors used a reconstitution assay by adding .sup.35S-I.kappa.B.alpha. (0.5 .mu.l) and purified **IKK complex** (5 nM) to a 5 .mu.l reaction mixture that also contains ATP (2 mM), TRAF6 (0.1 .mu.M), and column fractions (0.5-1 .mu.l). In most **IKK** activation assays, E1 (0.1 .mu.M) and Ub (0.1 mM) were also added to enhance the reaction, although they can be supplied from the wheat germ extracts used for in vitro translation of I.kappa.B.alpha.. As an assay for purification of TRIKA1, reaction mixtures also contain 0.5 .mu.g TRIKA2/FrIIa, a fraction eluted from Q-Sepharose with 0.2M NaCl. The reaction was carried out at 30.degree. C. for 1 hour, and then analyzed using a PhosphorImager (Molecular Dynamics) following SDS-PAGE.

Detail Description Paragraph - DETX (149):

[0173] TRAF6-dependent activation of **IKK** requires intermediary factors. To determine whether TRAF6 activates **IKK** directly, the inventors purified the **IKK complex** from unstimulated HeLa cells or from calf thymus through several chromatographic steps, including an ATP-affinity column step. The purified **IKK complex** from HeLa cells contains IKK.alpha., IKK.beta., and NEMO, and can be activated directly by MEKK1 (Lee et al., 1998). The ability to purify this latent **IKK complex** from HeLa cells allows the inventors to search for upstream factors required for its activation. One of the candidate upstream activators is TRAF6. However, unlike MEKK1, TRAF6 did not activate the purified **IKK complex** directly. In contrast, the crude extract from 293 cells can be activated by TRAF6, suggesting the existence of intermediary factors required for **IKK** activation by TRAF6 in the extract. As an initial step to identify

these factors, the inventors fractionated HeLa cytosolic extract (S100) on Q-Sepharose through step elution with increasing concentration of NaCl. Significantly, the activation of the **IKK complex** by TRAF6 requires at least two fractions, the unbound fraction (Fr.I) and the 0.1-0.2M NaCl eluate (Fr.IIa). Omission of either fraction abolishes **IKK** activation by TRAF6. The factor present in Fr.I is hereby referred to as TRAF6-Regulated **IKK** Activator 1 (TRIKA1), whereas the factor present in Fr.IIa is referred to as TRIKA2.

Detail Description Paragraph - DETX (152):

[0176] Recombinant Ubc13/Uev1A **complex** supports the activation of **IKK** by TRAF6. To verify that Ubc13 and Uev1A are indeed components of TRIKA1, the inventors expressed these proteins in E. coli and purified the recombinant proteins to apparent homogeneity with the aid of a hexa-histidine (His.sub.6) affinity tag. Recombinant Ubc13 and Uev1A form a heterodimer that migrates at approximately 45 kDa on a gel filtration column. In the presence of TRAF6, the recombinant Ubc13 and Uev1A activated **IKK** in a manner that correlated with the formation of the heterodimer. Furthermore, mutation of the active site Cys-87 of Ubc13 (C87A) abolished its ability to support **IKK** activation by TRAF6. Like the native TRIKA1, recombinant Ubc13/Uev1A stimulated I.kappa.B.alpha. phosphorylation by **IKK** at both serines 32 and 36. The inventors also tested whether other E2s could support TRAF6-mediated **IKK** activation. Among several E2s tested, only Ubc13/Uev1A was capable of activating **IKK** together with TRAF6. These results confirm that Ubc13/Uev1A is the TRIKA1 that links TRAF6 to **IKK** activation.

Detail Description Paragraph - DETX (155):

[0179] TRAF6 facilitates the assembly of K63-linked polyUb chains in conjunction with Ubc13/Uev1A. Previous studies have shown that Ubc13 and Mms2, a yeast homolog of Uev1A, form an E2 **complex** to catalyze the synthesis of unique polyUb chains linked through lysine-63 (K63) of ubiquitin (Hofmann and Pickart, 1999). This finding, together with the inventors' present results that this E2 **complex** is involved in **IKK** activation by TRAF6, raises the possibility that TRAF6 may also be involved in ubiquitination. In fact, TRAF6 contains a RING finger domain and five repeats of zinc fingers at its N-terminus (Cao et al., 1996). Several other RING finger proteins have previously been shown to function as ubiquitin ligases in polyubiquitination (Lorick et al., 1999). To test whether TRAF6 has a role in ubiquitination, the inventors carried out an in vitro ubiquitination assay in the presence of E1, Ubc13/Uev1A (as an E2), ATP, and Ub. When all components were present, polyUb chains synthesis was readily detectable with an Ub-specific antibody. In the absence of any of the components, no significant polyubiquitination was observed. (The Ub.sub.2 is principally a contaminant in the commercial Ub). Inclusion of an Ub mutant (KO) in which all seven lysines were mutated to arginine prevented polyUb chain formation. Restoration of a lysine at position 48 (K48) on an otherwise lysine-less background was not sufficient to restore polyubiquitination. In contrast, restoration of a lysine at position 63 (K63) restored polyubiquitination. These results show that TRAF6 facilitates the synthesis of polyUb chains through K63 rather than K48. In this regard, TRAF6

is an Ub ligase (E3) that partners with Ubc13/Uev1A (E2). The inventors also found that TRAF2 functioned together with Ubc13/Uev1A to catalyze the synthesis of K63-linked polyUb chains, consistent with the obligatory role of Ubc13/Uev1A in NF-.kappa.B activation by TNF.alpha. and TRAF2 (FIG. 2).

Detail Description Paragraph - DETX (160):

[0184] This invention reports, the purification and identification of a protein **complex** that links **IKK** to its upstream activator TRAF6. This **complex** is composed of Ubc13 and Uev1A (Mms2), a dimeric ubiquitin-conjugating enzyme previously shown to catalyze the synthesis of K63-linked polyubiquitin chains (Hofmann and Pickart, 1999).

Detail Description Paragraph - DETX (167):

[0189] Kinase Assays. **IKK** activity was measured in a reconstituted system (10 .mu.l) containing an ATP buffer (50 mM Tris-HCl, pH 7.5, 5 mM MgCl.sub.2, 2 mM ATP), .sup.35S -I.kappa.BA (11), purified **IKK complex** (5 nM), E1 (50 nM), Ubc13/Uev1A (0.3 .mu.M), TRAF6 (0.1 .mu.M), Ub (50 .mu.M), and various amounts of partially purified or immunopurified TRIKA2/TAK1 **complex**. Following incubation at 30.degree. C. for 1 hour, the reaction products were resolved by SDS-PAGE and analyzed using a PhosphorImager.

Detail Description Paragraph - DETX (168):

[0190] To assay the activity of TAK1, the kinase **complex** immobilized on the anti-TAB2 beads was incubated with E1, Ubc13/Uev1A, TRAF6, Ub and the ATP buffer at room temperature for 1 hour under conditions similar to those used for **IKK** activation (see above). The beads were then washed extensively with TBS (20 mM Tris-HCl, pH 7.5, 150 mM NaCl) plus 0.5% NP40, followed by incubation with recombinant MKK6 (1 .mu.M), .gamma.-.sup.32P-ATP (0.5 .mu.Ci/.mu.l), and a kinase buffer (50 mM Tris-HCl, pH 7.5, 0.5 mM DTT, 5 mM MgCl.sub.2, 50 .mu.M ATP). Phosphorylation of IKK.beta. was carried out similarly, except that IKK.beta. was preincubated with Nemo to form a **complex** which then serves as a substrate for TAK1. The reactions were carried out at 30.degree. C. for 1 hour and then analyzed by SDS-PAGE. For assays of JNK activity, the Ub-activated TAK1 beads were incubated with MKK6 (60 nM), JNK (0.1 .mu.M), .sup.35S-cJun or cJun (S63A/S73A, 1 .mu.l), and the ATP buffer. Phosphorylation of cJun was then determined based on its mobility shift on SDS-PAGE.

Detail Description Paragraph - DETX (172):

[0192] Identification of TRIKA2 as TAK1-TAB1-TAB2 **complex**. To facilitate the purification of TRIKA2 which might contain TAB2, the inventors prepared HeLa cell extracts in a buffer containing 0.5% NP40. From these extracts, the inventors used a TAB2-specific antibody to purify a **complex** containing stoichiometric amounts of TAK1, TAB1 and TAB2 as visualized by colloidal blue

staining and immunoblotting. This immunopurified **complex** contained a bona fide TRIKA2 activity in that it stimulated **IKK** only in the presence of both Ubc13/Uev1A and TRAF6 (together with other ubiquitination components including E1 and Ub).

Detail Description Paragraph - DETX (173):

[0193] To determine whether the TAK1/TAB1/TAB2 **complex** is indeed TRIKA2, the inventors transfected expression vectors harboring FLAG-tagged TAK1 and TAB2 cDNA, respectively, into 293 cells, and then immunopurified these proteins using a FLAG-specific antibody. In each case, the transfected protein associated with endogenous partners to form a TAK1/TAB1/TAB2 **complex,** which activated **IKK,** but only in the presence of both Ubc13/Uev1A and TRAF6. Importantly, a point mutation in the ATP binding domain of TAK1 (K63W) that abolished its kinase activity also abrogated its ability to stimulate **IKK**.

Detail Description Paragraph - DETX (174):

[0194] Since the catalytic activity of TAK1 was essential for **IKK** activation, the inventors examined whether TAK1 functioned as an **IKK** kinase (**IKKK**) to directly phosphorylate **IKK** at two specific serines (S177 and S181) in the activation loop. To activate endogenous TAK1, the inventors immunoprecipitated the TAK1 **complex** from HeLa cell extracts, which was then subjected to TRAF6-mediated ubiquitination reactions in the presence or absence of Ubc13/Uev1A. This TAK1 **complex** was then incubated with .gamma.-.sup.32p-ATP together with recombinant IKK.beta. proteins, which include a catalytically inactive mutant (K44M; to prevent IKK.beta. autophosphorylation) and a triple-point mutant in which two serines in the activation loop are also mutated (K44M/S177A/S181A). Significantly, the TAK1 kinase, once activated by Ubc13/Uev1A-mediated ubiquitination, was able to phosphorylate **IKK** specifically at serines 177 and 181. Thus, TAK1 is a ubiquitin-dependent kinase of IKK.beta..

Detail Description Paragraph - DETX (175):

[0195] TAK1 **complex** is both sufficient and necessary to activate **IKK** in conjuction with TRAF6 and Ubc13-Uev1A. To address the role of the individual components of the TAK1/TAB1/TAB2 **complex in IKK** activation, the inventors expressed TAK1, TAK1/TAB1, TAK1/TAB2 or co-expressed all three proteins in insect cells (Sf9) using the baculovirus expression system. These proteins were purified and then tested for **IKK** activation under the condition where the amount of TAK1 was equivalent. Only TAK1/TAB2 and TAK1/TAB1/TAB2 were able to stimulate **IKK** in the presence of Ubc13/Uev1A and TRAF6, suggesting that TAK1/TAB2 is the minimal sub-**complex** capable of activating **IKK**.

Detail Description Paragraph - DETX (176):

[0196] The above experiments were carried out using an **IKK complex** partially

purified from HeLa cell extracts (>60% pure). To determine whether the TAK1 **complex** could activate **IKK** in a reconstituted system using highly purified proteins, the inventors further purified the **IKK complex** to homogeneity by immunoprecipitation using a Nemo-specific antibody. The purified **IKK complex** contained exclusively IKK.alpha., IKK.beta. and Nemo/IKK.gamma. based on silver staining and immunoblotting. The inventors also obtained highly purified recombinant TAK1 **complex** (from Sf9 cells) and 35S-I.kappa.B.alpha.. Using these purified proteins, together with purified E1, E2 (Ubc13/Uev1A), and E3 (TRAF6), the inventors were able to reconstitute **IKK** activation by TRAF6 in a manner that was dependent on Ubc13/Uev1A (TRIKA1) and TAK1/TAB1/TAB2 (TRIKA2).

Detail Description Paragraph - DETX (177):

[0197] To determine whether the TAK1 **complex** is essential for **IKK** activation by TRAF6, the inventors immunodepleted this **complex** from 293 cell extracts using a TAB2 antibody. Removal of TAB2 from cell extracts abolished **IKK** activation by TRAF6, whereas immunoprecipitation with a control IgG antibody had no effect. Furthermore, addition of recombinant TAK1/TAB2 back into the depleted cell extracts restored **IKK** activation.

Detail Description Paragraph - DETX (178):

[0198] TAK1 is a ubiquitin-dependent kinase of the MKK-JNK pathway. Previous studies have shown that TRAF6 is essential for both **IKK** and JNK activation (Cao et al., 1996; Ishida et al., 1996; Lomaga et al., 1999; Naito et al., 1999), and that TAK1 can phosphorylate MKK6 (Ninomiya et al., 1999) which in turn activates the JNK/p38 kinase pathway (Raingeaud et al., 1996; Davis, 2000) The inventors' finding that **IKK** is activated by TAK1 in a TRAF6- and Ubc13/Uev1A-dependent manner raises the interesting possibility that the activation of MKK6 and subsequent activation of the JNK pathway might also be dependent on ubiquitin-activation of TAK1. Indeed, when the endogenous TAK1 **complex** was subjected to ubiquitination by TRAF6 and Ubc13/Uev1A, it was activated to phosphorylate MKK6. The Ub-activated TAK1 kinase specifically phosphorylates MKK6 at Ser-207 and Thr-211 in the activation loop (Raingeaud et al., 1996; Davis, 2000) allowing MKK6 to stimulate the kinase activity of JNK, which phosphorylates c-Jun at serines 63 and 73. In further support of the role of Ub in MKK/JNK activation, addition of methylated ubiquitin (MeUb), which blocks polyubiquitination (Hershko et al., 1985) to cell extracts abolished the activation of both JNK and **IKK** by TRAF6. Thus, Ub-activation of TAK1 provides a unifying mechanism for coordinate activation of the **IKK** and JNK pathways.

Detail Description Paragraph - DETX (179):

[0199] TRAF6 and Ubc13/Uev1A catalyze the synthesis of a unique polyubiquitin chain linked through lysine-63 (K63) of Ub, and the formation of such a chain is essential for **IKK** activation via a mechanism that does not involve proteasomal degradation (Deng et al., 2000) To determine whether the

formation of K63 chains is also important for TAK1 activation, the inventors subjected the immunopurified TAK1 **complex** to ubiquitination reactions in the presence of various Ub mutants (FIG. 5). Strikingly, a point mutation at position 63 from lysine to arginine (R63) completely abolished the ability of Ub to stimulate TAK1 activity (FIG. 5). Conversely, restoration of a single lysine at position 63 (K63) on an otherwise zero lysine background rescued the ability of Ub to activate TAK1. Thus, a lysine at position 63 is both necessary and sufficient for Ub to activate TAK1, most likely through the synthesis of K63-linked polyUb chains.

Detail Description Paragraph - DETX (181):

[0201] It has been suggested that oligomerization of TRAF6 through its C-terminal TRAF domain leads to the activation of the **IKK** and JNK pathways (Baud et al., 1999). To determine whether oligomerization of TRAF6 triggers its ubiquitination, the inventors sought to create a cell line in which the activation of **IKK** is controlled by the inducible oligomerization of TRAF6. The inventors replaced the C-terminal TRAF domain of TRAF6 with a fragment of the bacterial gyrase B, which dimerizes in the presence of coumermycin A (Farrar, 1996) (FIG. 6). The chimeric construct (T6RZC) was transfected into 293 cells to establish a stable line in which I.kappa.B.alpha. was rapidly degraded upon the addition of coumermycin A. Concomitant with the degradation of I.kappa.B.alpha., a ladder of polyubiquitinated T6RZC accumulated. Notably, while treatment of cells with coumermycin A or IL-1.beta. induced the rapid degradation of I.kappa.B.alpha., no evidence of T6RZC or TRAF6 degradation was observed during the same time course. Interestingly, deletion of a coil-coiled region (residues 292-358) in TRAF6 (T6RZ) abolishes its ubiquitination as well as its ability to stimulate I.kappa.B.alpha. degradation, suggesting a correlation between the ability of TRAF6 to be ubiquitinated and its ability to stimulate the **IKK** pathway. Following ubiquitination, TRAF6 led to the activation of **IKK** in the presence of the TAK1 **complex** without further requirements for additional ubiquitination enzymes such as E1 and Ubc13/Uev1A. The polyUb chains on T6RZC were apparently K63-linked, since it was polyubiquitinated by Ub mutants containing K63 but not R63.

Detail Description Paragraph - DETX (182):

[0202] The invention also reports the purification and identification of TRIKA2, which turns out to be composed of TAK1, TAB1 and TAB2, a protein kinase **complex** previously implicated in **IKK** activation through an unknown mechanism (Ninomiya et al., 1999; Takaesu et al., 2000) Furthermore, the data indicates that the TAK1 kinase **complex** phosphorylates and activates **IKK** in a manner that depends on TRAF6 and Ubc13/Uev1A. Moreover, the activity of TAK1 to phosphorylate MKK6, which activates the JNK/p38 kinase pathway, is directly regulated by K63-linked polyubiquitination. Evidence that TRAF6 itself is conjugated by the K63 polyUb chains is provided herein. These results indicate that ubiquitination plays an important regulatory role in stress response pathways, including those of **IKK** and JNK. The data strongly suggest that TAK1 is an **IKKK** that phosphorylates and activates **IKK** in the TRAF6 pathway. Futhermore, the results clearly demonstrate a crucial role of K63-linked

polyubiquitination in TAK1 activation.

Claims Text - CLTX (2):

1. A method of screening for modulators of **IKK** and JNK activation comprising: (a) providing a Ubc13/Uev1A **complex** and TRAF6 or TRAF2; (b) contacting said **complex** and TRAF6 or TRAF2 with a candidate modulator substance in the presence of E1, a plurality of ubiquitin molecules and ATP; (c) determining the formation of free polyubiquitin chains, wherein a change in poly-ubiquitin formation in the presence of said candidate modulator, as compared with poly-ubiquitin formation in the absence of said candidate modulator, indicates that said candidate modulator is an modulator of **IKK** and JNK activation.

Claims Text - CLTX (17):

16. A method of screening for modulators of **IKK** and JNK activation comprising: (a) providing a Ubc13/Uev1A **complex,** TRAF6 or TRAF2; (b) contacting TAB1/TAB2/TAK1 **complex,** Ubc13/Uev1A **complex,** TRAF6 or TRAF2 with a candidate modulator substance in the presence of E1, a plurality of ubiquitin molecules and ATP; (c) determining the polyubiquitination of TRAF6 or TRAF2, wherein a change in the phosphorylation state of **IKK** or MKK in the presence of said candidate modulator, as compared with the phosphorylation state of **IKK** or MKK in the absence of said candidate modulator, indicates that said candidate modulator is an modulator of **IKK** and JNK activation.

Claims Text - CLTX (55):

54. A method of screening for modulators of **IKK** and JNK activation comprising: (a) providing TAK1, TAB 1, TAB2, TRAF6 and **IKK complex**; (b) contacting TAK1, TAB1, TAB2, TRAF6 and **IKK complex** with a candidate modulator substance in the presence of E1, Ubc13/Uev1A, a plurality of ubiquitin molecules and ATP; (c) determining the phosphorylation state of I.kappa.B and MKK, wherein a change in the phosphorylation state of I.kappa.B and MKK in the presence of said candidate modulator, as compared with the phosphorylation state of I.kappa.B and MKK in the absence of said candidate modulator, indicates that said candidate modulator is an modulator of **IKK** and JNK activation.

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ABSTRACT:

The present invention relates to the discovery of novel protein-protein interactions that are involved in mammalian physiological pathways, including physiological disorders or diseases. Examples of physiological disorders and diseases include non-insulin dependent diabetes mellitus (NIDDM), neurodegenerative disorders, such as Alzheimer's Disease (AD), and the like. Thus, the present invention is directed to complexes of these proteins and/or their fragments, antibodies to the complexes, diagnosis of physiological generative disorders (including diagnosis of a predisposition to and diagnosis of the existence of the disorder), drug screening for agents which modulate the interaction of proteins described herein, and identification of additional proteins in the pathway common to the proteins described herein.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is related to U.S. provisional patent application Serial No. 60/259,572, filed on Jan. 4, 2001, incorporated herein by reference, and claims priority thereto under 35 USC .sctn.119(e).

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (33):

[0031] NFkB activity is controlled by protein-protein interactions that alter its subcellular localization (Karin and Ben-Neriah, 2000; Karin, 1999; Mercurio and Manning, 1999). In unstimulated cells, NFkB is inactive and sequestered in the cytoplasm due to its interaction with IkappaB (IkB), which masks the NFkB nuclear localization signal. Upon stimulation, IkB is phosphorylated, which targets it for ubiquitination and proteasome-mediated degradation. Disruption of the IkB/NFkB **complex** frees NFkB to enter the nucleus and activate transcription of proinflammatory genes. A key step in NFkB activation is the initial phosphorylation of IkB; this is accomplished by IkB-kinase (**IKK**) family members, which are in turn responsive to signals from cell surface receptors for factors such as TNF-alpha and IL-1. Clearly, identifying all of the proteins involved in NFkB activation is necessary to understand the process by which extracellular signals are transduced into NFkB-mediated transcriptional responses. Furthermore, identification of these proteins will increase the repertoire of potential targets for therapeutic intervention in the treatment of diseases due to defects involving NFkB activation, such as arthritis, asthma, and cancer.

Summary of Invention Paragraph - BSTX (34):

[0032] IkB kinases (**IKKs**) are responsible for signal-induced phosphorylation IkB, leading to IkB degradation and activation of NFkB. These proteins appear to function as a **complex of IKK** family members, and may interact with other cellular factors as well. Consequently, the **IKKs** and proteins with which they interact are potential targets of anti-inflammatory (and other) drugs. Four **IKKs** [IKK-alpha (**IKKa**), **IKK**-beta (**IKKb**), **IKK**-gamma (**IKKg**), and inducible **IKK** (**IKK-i**)] have been identified (reviewed in Karin and Ben-Neriah, 2000; Karin, 1999; Mercurio and Manning, 19998-10). These proteins were used in yeast two-hybrid assays to identify **IKK**-interacting proteins.

Summary of Invention Paragraph - BSTX (36):

[0034] The second **IKKb** interactor is a subunit of translation initiation factor 3 (EIF3S10). EIF3S10 is the largest subunit of the EIF3 **complex**. It contains a so-called PCI domain that is found in other proteins also found in large complexes, such as components of the COP9 signalosome (Scholler et al., 1997). The interaction of EIF3S20 with **IKKb** suggests that phosphorylation of the translation machinery may be part of the inflammatory response. This possibility is further supported by our identification of interactions between MAPKAP-K3, a protein kinase involved in the inflammatory response, and the translation-associated proteins ERF-2, SUI1, and PAIP1.

Detail Description Paragraph - DETX (19):

[0082] As shown above, **IKKb** interacts with LDHM to form a **complex**. A **complex** of the two proteins is prepared, e.g., by mixing purified preparations of each of the two proteins. If desired, the protein **complex** can be stabilized by cross-linking the proteins in the **complex,** by methods known to those of

skill in the art. The protein **complex** is used to immunize rabbits and mice using a procedure similar to that described by Harlow et al. (1988). This procedure has been shown to generate Abs against various other proteins (for example, see Kraemer et al., 1993).

Detail Description Paragraph - DETX (20):

[0083] Briefly, purified protein **complex** is used as immunogen in rabbits. Rabbits are immunized with 100 .mu.g of the protein in complete Freund's adjuvant and boosted twice in three-week intervals, first with 100 .mu.g of immunogen in incomplete Freund's adjuvant, and followed by 100 .mu.g of immunogen in PBS. Antibody-containing serum is collected two weeks thereafter. The antisera is preadsorbed with **IKKb** and LDHM, such that the remaining antisera comprises antibodies which bind conformational epitopes, i.e., **complex**-specific epitopes, present on the **IKKb**-LDHM **complex** but not on the monomers.

Detail Description Paragraph - DETX (26):

[0087] Spleens are removed from immune mice and a single-cell suspension is prepared (Harlow et al., 1988). Cell fusions are performed essentially as described by Kohler et al. (1975). Briefly, P3.65.3 myeloma cells (American Type Culture Collection, Rockville, Md.) or NS-1 myeloma cells are fused with immune spleen cells using polyethylene glycol as described by Harlow et al. (1988). Cells are plated at a density of 2.times.10.sup.5 cells/well in 96-well tissue culture plates. Individual wells are examined for growth, and the supernatants of wells with growth are tested for the presence of **IKKb**/LDHM **complex**-specific antibodies by ELISA or RIA using **IKKb**/LDHM **complex** as target protein. Cells in positive wells are expanded and subcloned to establish and confirm monoclonality.

Detail Description Paragraph - DETX (27):

[0088] Clones with the desired specificities are expanded and grown as ascites in mice or in a hollow fiber system to produce sufficient quantities of antibodies for characterization and assay development. Antibodies are tested for binding to **IKKb** alone or to LDHM alone, to determine which are specific for the **IKKb**/LDHM **complex** as opposed to those that bind to the individual proteins.

Detail Description Paragraph - DETX (32):

[0091] The present invention is useful in screening for agents that modulate the interaction of **IKKb** and LDHM. The knowledge that **IKKb** and LDHM form a **complex** is useful in designing such assays. Candidate agents are screened by mixing **IKKb** and LDHM (a) in the presence of a candidate agent, and (b) in the absence of the candidate agent. The amount of **complex** formed is measured for each sample. An agent modulates the interaction of **IKKb** and LDHM if the amount of **complex** formed in the presence of the agent is greater than (promoting the

interaction), or less than (inhibiting the interaction) the amount of **complex** formed in the absence of the agent. The amount of **complex** is measured by a binding assay, which shows the formation of the **complex**, or by using antibodies immunoreactive to the **complex**.

Detail Description Paragraph - DETX (33):

[0092] Briefly, a binding assay is performed in which immobilized **IKKb** is used to bind labeled LDHM. The labeled LDHM is contacted with the immobilized **IKKb** under aqueous conditions that permit specific binding of the two proteins to form a **IKKb**/LDHM **complex** in the absence of an added test agent. Particular aqueous conditions may be selected according to conventional methods. Any reaction condition can be used as long as specific binding of **IKKb**/LDHM occurs in the control reaction. A parallel binding assay is performed in which the test agent is added to the reaction mixture. The amount of labeled LDHM bound to the immobilized **IKKb** is determined for the reactions in the absence or presence of the test agent. If the amount of bound, labeled LDHM in the presence of the test agent is different than the amount of bound labeled LDHM in the absence of the test agent, the test agent is a modulator of the interaction of **IKKb** and LDHM.

Detail Description Paragraph - DETX (37):

[0094] In addition to the in vitro method described in Example 16, an in vivo assay can also be used to screen for agents which modulate the interaction of **IKKb** and LDHM. Briefly, a yeast two-hybrid system is used in which the yeast cells express (1) a first fusion protein comprising **IKKb** or a fragment thereof and a first transcriptional regulatory protein sequence, e.g., GAL4 activation domain, (2) a second fusion protein comprising LDHM or a fragment thereof and a second transcriptional regulatory protein sequence, e.g., GAL4 DNA-binding domain, and (3) a reporter gene, e.g., .beta.-galactosidase, which is transcribed when an intermolecular **complex** comprising the first fusion protein and the second fusion protein is formed. Parallel reactions are performed in the absence of a test agent as the control and in the presence of the test agent. A functional **IKKb**/LDHM **complex** is detected by detecting the amount of reporter gene expressed. If the amount of reporter gene expression in the presence of the test agent is different than the amount of reporter gene expression in the absence of the test agent, the test agent is a modulator of the interaction of **IKKb** and LDHM.

Claims Text - CLTX (2):

1. An isolated protein **complex** comprising two proteins, the protein **complex** selected from the group consisting of: (i) a **complex** of a first protein and a second protein; (ii) a **complex** of a fragment of said first protein and said second protein; (iii) a **complex** of said first protein and a fragment of said second protein; and (iv) a **complex** of a fragment of said first protein and a fragment of said second protein, wherein said first and second proteins are selected from the group consisting of: (a) said first protein is **IKKb** and said

second protein is selected from the group consisting of LDHM, EIF3S10, SLAP2, KIAA0614, SART-1 and GBDR1; (b) said first protein is **IKKa** and said second protein is GBDR1; (c) said first protein is **IKKg** and said second protein is TRAF; and (d) said first protein is **IKK-i** and said second protein is selected from the group consisting of NUMA1, SPA-1 and PN13730.

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ABSTRACT:

The present invention relates to nucleotide sequences encoding a modulator of NF-.kappa.B, and to the polypeptides encoded by the nucleotide sequences. In particular, the invention relates to nucleotide sequences and the polypeptides encoded thereby, wherein the polypeptides are involved in the response to NF-.kappa.B-activating stimuli, including HTLV-1 Tax, LPS, PMA and IL-1. The invention also relates to antibodies to the modulator of NF-.kappa.B, methods of detecting modulator of NF-.kappa.B using the antibodies, methods of treatment associated with NF-.kappa.B activation and to methods of identifying compounds which modulate the activity of the modulator of NF-.kappa.B.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (7):

[0005] The recent description of a high molecular weight cytoplasmic **complex** able to phosphorylate I.kappa.B.alpha. on serines 32 and 36 (Chen et al., 1996; Lee et al., 1997) has prompted intense studies, which culminated a few months ago with the cloning of two kinases, named **IKK-1 and IKK-2,** or

IKK.alpha. and IKK.beta. (Didonato et al., 1997; Mercurio et al., 1997; Regnier et al., 1997; Woronicz et al., 1997; Zandi et al., 1997). Two approaches were used to this end: one involved biochemical purification from a cytoplasmic extract derived from TNF-treated HeLa cells (Didonato et al., 1997; Mercurio et al., 1997; Zandi et al., 1997), while the other used a 2-hybrid screen using as a bait NIK, a protein kinase previously shown to be involved in TNF- and IL-1-induced NF-.kappa.B activation (Regnier et al., 1997; Woronicz et al., 1997). The cloned kinases were postulated to directly phosphorylate serines 32 and 36 of I.kappa.B.alpha., although this has not been formally demonstrated. The reason for this uncertainty is that all kinase assays reported so far rely on immunoprecipitation of transfected or in vitro translated **IKK,** therefore leaving open the possibility that the "true" I.kappa.B kinase is coprecipitated together with **IKK** and the rest of the high molecular weight **complex**. Immunoprecipitation of one kinase from extracts of cells transfected with the two kinases results in the coprecipitation of the second kinase, and a more detailed study has demonstrated that hetero-association was favored over homo-association. The sequence of **IKK-1 and IKK-2** has revealed two interesting features: a leucine zipper and a helix-loop-helix motif. Deletion of the LZ in one of the kinases results in the abrogation of coimmunoprecipitation with either itself or the other kinase, and a strong reduction in the resulting kinase activity. However it is unclear whether the LZ motif is required for direct interaction between the kinase subunits or between the kinase(s) and some other component of the **complex**. Deletion of the HLH motif leaves the coimmunoprecipitation of the two kinases intact, but strongly reduces the resulting kinase activity. In the assays used in the above mentioned papers, transfected **IKK-2** seems to exhibit a stronger basal kinase activity when compared to **IKK-1** (Mercurio et al., 1997; Zandi et al., 1997). Zandi et al. (Zandi et al., 1997) also observed that cotranslation of the two kinases in wheat germ extracts resulted in no I.kappa.B kinase activity, suggesting that either post-translational modifications or additional components of the **complex** (or both) are required. Cotranslation of the two kinases in wheat germ extracts precluded their association. One possibility is that the kinase subunits need to be incorporated into the 600-800 kD **complex** in order to be fully active, and that some critical components of the **complex** are absent in wheat germ extracts. In any case all these data emphasize the importance of identifying additional components of the **complex**.

Brief Description of Drawings Paragraph - DRTX (17):

[0031] FIG. 6. NEMO is associated with an inducible endogenous I.kappa.B.alpha. kinase activity. Rat-1 or 5R cells were treated for 5 minutes with or without TNF-.alpha. (10 ng/ml). Cytoplasmic extracts were immunoprecipitated with either preimmune serum (P.I.), anti-**IKK**-1 antibody (anti-**IKK**-1) or NEMO antiserum (anti-NEMO) and specific I.kappa.B.alpha. kinase activity was determined by an in vitro immune **complex** kinase assay with GST-I.kappa.B.alpha. (1-72) wild type or GST-I.kappa.B.alpha. (1-72) S32A/S36A mutant protein as substrates.

Brief Description of Drawings Paragraph - DRTX (19):

[0033] A. Gel filtration analysis of NEMO and I.kappa.B kinase **complex** in Rat-1 and 5R cells. S100 extracts were prepared as described in Materials and Methods and fractionated through a Superose 6 column. Fractions were analyzed by Western blotting, using antibodies specific for **IKK-1** or NEMO. Analysis of NF-.kappa.B/I.kappa.B elution, using an anti-relA antibody is also shown. To demonstrate identical elution of Rat-1 and 5R extracts, the protein profile from each fraction was analyzed by silver staining (Upper pannel).

Detail Description Paragraph - DETX (157):

[0193] In accordance with the present invention, a mutant cell line, 5R, was originally isolated as a cellular flat variant of Rat-1 fibroblasts transformed by the Tax protein of human T-cell leukemia virus type 1 (HTLV-1). Tax is known to activate transcription from the HTLV-1 long terminal repeat, to cause permanent activation of many cellular transcription factors including NF-.kappa.B and to give rise to cellular transformation (for a review, see Yoshida et al., 1995). 5R cells carry a recessive cellular mutation which abolishes Tax-induced constitutive NF-.kappa.B activity, therefore providing a potential mean of identifying a critical molecule involved in Tax-mediated NF-.kappa.B activation. Interestingly, 5R cells were found to be resistant to multiple NF-.kappa.B activating stimuli besides Tax, suggesting they carried a mutation at a converging regulatory step. 5R cells were used for a genetic complementation approach for the following reasons. First, as the screen was based on the NF-.kappa.B-dependent expression of a drug resistance gene, the presence of Tax would ensure restoration of a permanent high NF-.kappa.B activity following complementation. Second, Rat-1-derived cells grow well in the presence of a high NF-.kappa.B activity. Third, 5R cells are expected to show a transformed phenotype following complementation. Here the genetic complementation of 5R cells is described. By infection with a cDNA expression library cloned into a retroviral vector, complemented clones derived from 5R cells were obtained and expression of the cloned gene, nemo, also complements the defect in the 10.3E2 cell line and show that NEMO is part of the high molecular weight **IKK complex** and is required for its formation.

Detail Description Paragraph - DETX (190):

[0224] Since NEMO appears to be critically involved in NF-.kappa.B activation by a large set of stimuli and complements cells defective in I.kappa.B phosphorylation, an attractive possibility would be that it constitutes a subunit of the 600-800 kD kinase **complex** that phosphorylates I.kappa.B. Therefore, it was investigated whether NEMO is associated with the inducible I.kappa.B kinase activity (FIG. 6). To demonstrate this point immune **complex** kinase assays were conducted on Rat-1 or 5R cells. The antiserum against NEMO immunoprecipitated a specific endogenous I.kappa.B.alpha. kinase activity from wild type cells stimulated with TNF-.alpha.. Absense of kinase activity in NEMO-immunoprecipitates from 5R cells and lack of phosphorylation of a mutant I.kappa.B.alpha. polypeptide (S32A, S36A) established the specificity of the antiserum and kinase activity, respectively. Thus, NEMO is associated with an inducible endogenous I.kappa.B.alpha. kinase activity. As reported previously, an anti-**IKK**-1 antibody brought down a specific I.kappa.B.alpha. kinase activity from wild type cells stimulated with

TNF-.alpha. for 5 minutes. Interestingly, no inducible I.kappa.B.alpha. kinase activity was observed in **IKK-1** precipitates from SR cell extracts.

Detail Description Paragraph - DETX (191):

[0225] In order to confirm that NEMO is an integral part of the I.kappa.B kinase **complex,** and to determine whether it is stably associated with it before stimulation, S100 extracts were prepared from Rat-1 cells and fractionated on a Superose 6 gel filtration column. Elution of the I.kappa.B kinase, monitored with an anti-**IKK**-1 antibody, was mostly observed in fractions containing proteins of 600 to 800 kD, as previously reported (FIG. 7A). When NEMO elution was examined, an identical profile was obtained. Immunoprecitation of the NEMO-containing fractions with an anti-NEMO antibody allowed us to co-immunoprecipitate **IKK-1** (FIG. 7B). NEMO is therefore a stable component of the 600-800 kDa I.kappa.B kinase **complex**.

Detail Description Paragraph - DETX (192):

[0226] Quite remarkably, when 5R extracts were analyzed with the **IKK-1** antibody, the elution peak appeared shifted toward fractions containing proteins of 300-450 kD instead of 600-800 kD (FIG. 7A). Since the overall elution profile, as checked either by silver staining (FIG. 7A, top panel) or by Western blotting against RelA (FIG. 7A, bottom panel) or p105 (data not shown), was identical between Rat-1 and 5R, this observation demonstrated the requirement of NEMO for building a high molecular weight I.kappa.B kinase **complex**. Moreover, the absence of I.kappa.B kinase activity in 5R cells after stimulation (see above) indicates that the lower molecular weight kinase **complex** is refractory to activation.

Detail Description Paragraph - DETX (195):

[0229] Since NEMO is part of the I.kappa.B kinase **complex,** direct interactions with known components of the **complex** were examined, namely the two catalytic subunits **IKK-1 and IKK-2**. An in vitro analysis was conducted using .sup.35S-labeled proteins translated in wheat germ extracts (WGE). After co-translation of VSV-**IKK**-2 and NEMO, followed by anti-VSV imunoprecipitation, NEMO was readily detected in the immunoprecipitate (FIG. 7D). The converse experiment, using NEMO plus VSV-**IKK**-2 and imunoprecipitating with anti-NEMO allowed the detection of VSV-**IKK**-2 in the immunoprecipitate. Interestingly, such an interaction could barely be observed with **IKK-1,** suggesting a potential functional divergence between the two **IKKs**.

Detail Description Paragraph - DETX (199):

[0233] The next question concerned the actual function of NEMO. Since this molecule appears to be involved in all tested NF-.kappa.B activating pathways, an obvious possibility was that it constituted one subunit of the high molecular weight I.kappa.B kinase **complex**. Three arguments are in favor of

this hypothesis: First, immunoprecipitation of NEMO from Rat-1 cells pulled down a bonafide I.kappa.B.alpha. kinase activity, specific for the 2 N-terminal serines. Second, NEMO elutes as a 600-800 kDa peak from a gel filtration column performed on extracts from unstimulated Rat-1 cells, as does **IKK-1**. Third, immunoprecipitation of NEMO from Rat-1 fractions ranging from 600 to 800 KDa brings down **IKK-1**.

Detail Description Paragraph - DETX (200):

[0234] The possible interaction of NEMO with the 2 catalytic subunits of the **complex** was tested, **IKK-1 and IKK-2**. In vitro cotranslation of **IKK-2** and NEMO in wheat germ extract followed by immunoprecipitation demonstrated that the two proteins could interact with each other. In contrast an interaction between NEMO and **IKK-1** could barely be detected under these conditions. NEMO can also form homodimers.

Detail Description Paragraph - DETX (201):

[0235] **IKK-1** can be detected in a 300-450 kD **complex** in 5R cells, therefore indicating that NEMO is required for the formation of a 600-800 kDa functional **IKK complex,** and probably plays a role as a structural component of this **complex**. It was unexpected that two independently isolated mutant cell lines could be complemented by the same cDNA. The selection for LPS-unresponsive derivatives of 70Z/3 yielded several types of mutant cell lines, but only 10.3E2 was also unresponsive to other NF-.kappa.B activating stimuli, and the fact that it grows faster than the wild-type 70Z/3 probably facilitated its isolation. In Tax transformed Rat-1 cells, SR was the only NF-.kappa.B defective cellular revertant which could be isolated. Mutating the nemo gene might be the only means of knocking out NF-.kappa.B activation by a single gene mutation.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030059791 A1

TITLE: Method for evaluating DNA probes position on substrate

PUBLICATION-DATE: March 27, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 083550

DATE FILED: February 27, 2002

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	2001-053465	2001JP-2001-053465	February 28, 2001
JP	2002-022682	2002JP-2002-022682	January 31, 2002

US-CL-CURRENT: 435/6, 435/287.2 , 702/20

ABSTRACT:

An oligonucleotide array comprising an array of multiple oligonucleotides with different base sequences fixed onto known and separate positions on a support substrate, wherein said oligonucleotides are biological stress related genes or complementary sequence chains to the said genes, and the said oligonucleotides are classified according to their gene functions, wherein the fixation region on the support substrate is divided into the said classification.

---------- KWIC ---------

Detail Description Table CWU - DETL (18):

18TABLE 18 J03171 Human interferon-a1pha receptor (HuIFN-alpha-Rec) mRNA, complete cds X77722 H. sapiens mRNA for interferon alpha/beta receptor V00547 Human messenger RNA for fibroblast (beta) interferon X13274 Human mRNA for interferon IFN-gamma J03143 Human interferon-gamma receptor mRNA, complete cds U05875 Human clone pSK1 interferon gamma receptor accessory factor-1 (AF-1) mRNA, complete cds X02669 Human mRNA for type 1 interferon-omega 1. Y08915 Immunoglobulin (CD79A) binding protein 1 X57025 Human IGF-I mRNA for insulin-like growth factor I X04434 Human mRNA for insulin-like growth factor I receptor J03242 Human insulin-like growth factor

II mRNA, complete cds J03528 Human cation-independent mannose 6-phosphate receptor mRNA; insulin-like growth factor II receptor M31145 Human insulin-like growth factor binding protein mRNA, complete cds M35410 Human insulin-like growth factor binding protein 2 (IGFBP2) mRNA M31159 Human growth hormone-dependent insulin-like growth factor-binding protein mRNA, complete cds M62403 Human insulin-like growth factor binding protein 4 (IGFBP4) mRNA, complete cds AF055033 Homo sapiens clone 24645 insulin-like growth factor binding protein 5 (IGFBP5) mRNA, complete cds M62402 Human insulin-like growth factor binding protein 6 (IGFBP6) mRNA, complete cds S75725 prostacyclin-stimulating factor [human, cultured diploid fibroblastcells, mRNA, 1124 nt]. AF044195 Homo sapiens IkappaB kinase **complex** associated protein (IKAP) mRNA, complete cds; IKKAP2 AF080158 Homo sapiens IkB kinase-b (**IKK**-beta) mRNA, **IKK2**/beta; **IKK2** AF074382 Homo sapiens IkB kinase gamma subunit (**IKK**-gamma) mRNA, NLK M57627 Human interleukin 10 (IL10) mRNA, complete cds U00672 Human interleukin-10 receptor mRNA, complete cds Z17227 Homo sapiens mRNA for transmebrane receptor protein M57765 Human interleukin 11 mRNA, complete cds

PGPUB-DOCUMENT-NUMBER: 20030059419

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030059419 A1

TITLE: Identification of novel substrate I-TRAF of IKK-i kinase

PUBLICATION-DATE: March 27, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 298402

DATE FILED: November 18, 2002

RELATED-US-APPL-DATA:

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child 09582397 US

parent a-371-of-international PCT/JP99/05916 19991026 WO UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	10-304085/1998	1998JP-10-304085/1998	October 26, 1998

US-CL-CURRENT: 424/94.5, 435/194 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

Novel IkB kinase IKK-i which is a novel serine/threonine kinase capable of activating a transcription factor NF-kB which inhibits the expression of various genes relating to immune response; a gene encoding the same; and medicinal compositions containg the same.

---------- KWIC ---------

Detail Description Paragraph - DETX (46):

[0069] The TRAF molecule is activated by forming a **complex** with a receptor as a result of stimulation by a ligand. However, when activation does not

occur, activation is thought to be negatively regulated by binding with I-TRAF in the cytoplasm. Consequently, **IKK-i** is thought to be involved indirectly in the activation of the TRAF molecule by phosphorylation of the I-TRAF.

PGPUB-DOCUMENT-NUMBER: 20030054999

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030054999 A1

TITLE: Anti-inflammatory compounds and uses thereof

PUBLICATION-DATE: March 20, 2003

INVENTOR-INFORMATION:

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APPL-NO: 09/ 847946

DATE FILED: May 2, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60201261 20000502 US

US-CL-CURRENT: 514/15, 514/16 , 514/17 , 530/326 , 530/327 , 530/328 , 530/329

ABSTRACT:

The present invention provides anti-inflammatory compounds, pharmaceutical compositions thereof, and methods of use thereof for treating inflammatory disorders. The present invention also provides methods of identifying anti-inflammatory compounds and methods of inhibiting NF-.kappa.B-dependent target gene expression in a cell.

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 60/201,261 filed May 2, 2000 and to U.S. patent application Ser. No. 09/643,260 filed Aug. 22, 2000, the entire contents of each of which are incorporated herein by reference.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (6):

[0028] FIG. 5 depicts results from experiments indicating that a

cell-permeable peptide spanning the IKK.beta. NBD inhibits the IKK.beta./NEMO interaction, TNF.alpha.-induced NF-.kappa.B activation and NF-.kappa.B-dependent gene expression. (A) GST-pull-down analysis was performed using either GST-NEMO-in vitro translated IKK.beta. (upper panel) or GST-IKK.beta.-(644-756)-in vitro translated NEMO (lower panel). The assay was performed in the absence (no peptide) or presence of increasing concentrations (125, 250, 500 or 1000 .mu.M) of either mutant (MUT) or wild-type (WT) NBD peptide. (B) HeLa cells were incubated with either peptide (200 .mu.M) for the times indicated. Following lysis, the **IKK complex** was immunoprecipitated using anti-NEMO and the resulting immunoblot probed with anti-IKK.beta.. (C) HeLa cells were transfected for forty-eight hours with pBIIX-luciferase then incubated for two hours in the absence (control) or presence of mutant or wild-type NBD peptide (100 and 200 .mu.M of each). Subsequently the cells were either treated with TNF.alpha. (10 ng/ml) as indicated (left panel) or left untreated (right panel) for a further four hours after which NF-.kappa.B activation was measured by luciferase assay. (D) HeLa cells were incubated for three hours with increasing concentrations (50, 100 or 200 .mu.M) of each peptide followed by treatment for fifteen minutes with TNF.alpha. (10 ng/ml) as indicated (+). Following lysis, nuclear extracts were made and 10 .mu.g of protein from each sample was used for EMSA using a specific [.sup.32P]-labeled .kappa.B-site probe. (E) Primary HUVEC were pre-incubated for two hours with of wild-type (left) or mutant (right) NBD peptides (100 .mu.M) then stimulated with TNF.alpha. (10 ng/ml) for a further six hours. Control cells received no peptide. Cells were stained with either anti-E-selectin (H4/18) or a non-binding control antibody (K16/16) and expression was measured by FACS (FACSort, Becton Dickinson). The profiles show E-selectin staining in the absence (shaded) and presence (solid line) of TNF.alpha. and control antibody staining under the same conditions (dashed line, no TNF.alpha.; dotted line, TNF.alpha.).

Detail Description Paragraph - DETX (14):

[0048] As used herein, the term "I.kappa.B-kinase" or "I.kappa.B protein kinase" or "I.kappa.B-kinase **complex**" or "I.kappa.B protein kinase **complex" or "IKK**" refers to a kinase that phosphorylates I.kappa.Bs.

Detail Description Paragraph - DETX (146):

[0175] These results establish that the interaction domain lies between residues 44 and 86, a region including the first .alpha.-helix of NEMO. A mutant was therefore made in which .alpha.-helix up to the first coiled-coil domain was deleted (residues T50-L93; del..alpha.H). This mutant did not interact with IKK.beta.-(644-756) (FIG. 2B). Furthermore transfection studies using pBIIX-luciferase demonstrated that del..alpha.H inhibited TNF.alpha.-induced NF-.kappa.B activity (FIG. 2C) confirming previous reports that the COOH-terminus of NEMO which can not interact with IKK.beta., is a dominant-negative inhibitor of NF-.kappa.B (Mercurio et al., (1999) Mol. Cell. Biol. 19, 1526-1538; Rothwarf et al., (1998) Nature 395, 297-300). Taken together, FIGS. 1 and 2 show that the interaction between IKK.beta. and NEMO occurs via the COOH-terminus of IKK.beta. and the first a-helical region of NEMO. These findings suggest a model in which the NH2-terminus of NEMO anchors

it to the **IKK-complex** leaving the remainder of the molecule containing several protein:protein interaction domains free and accessible for interacting with upstream regulators of **IKK** function.

Detail Description Paragraph - DETX (153):

[0180] These results demonstrate that basal auto-phosphorylation and kinase activity of IKK.beta. is dependent on the ability of NEMO to associate with the kinase. One explanation for these observations may be that NEMO recruits a phosphatase to the **IKK-complex** that regulates basal IKK.beta. function by targeting the serine-rich region of the COOH-terminus. Inability to bind NEMO therefore prevents phosphatase recruitment and causes increased phosphorylation within this region.

Detail Description Paragraph - DETX (155):

[0182] An additional band representing a phosphorylated protein appeared only in the samples from TNF.alpha.-induced IKK.beta. (WT) and IKK.beta.-(1-744) transfected cells (FIG. 3F). The molecular weight of this protein (49 kDa) strongly suggests that it is endogenous NEMO associated with the precipitated **complex**. This is supported by the absence of the band in either precipitate (+/-TNF.alpha.) from IKK.beta.-(1-733) transfected cells. This protein has been identified as phosphorylated NEMO by dissociating the precipitated **complex** in SDS and re-immunoprecipitating [.sup.32P]-labeled NEMO using specific anti-NEMO antibodies. Induced phosphorylation of NEMO may therefore represent a further level of regulation of the activity of the **IKK complex**.

Detail Description Paragraph - DETX (168):

[0189] The relatively small size of the NBD makes it an attractive target for the development of compounds aimed at disrupting the core **IKK complex**. The relevance of this approach was investigated by designing cell-permeable peptides spanning the IKK.beta.NBD and determining their ability to dissociate the IKK.beta.-NEMO interaction.

Detail Description Paragraph - DETX (170):

[0191] The wild-type NBD peptide consisted of the region from T735 to E745 of IKK.beta. fused with a sequence derived from the third helix of the antennapedia homeodomain that has been shown to mediate membrane translocation (Derossi et al., (1994) J. Biol. Chem. 269, 10444-10450). The mutant was identical except that the tryptophan residues (W739 and W741) in the NBD were mutated to alanine. FIG. 5A shows that the NBD (WT) but not the mutant dose-dependently inhibited in vitro pull-down of [.sup.35S]-labeled IKK.beta. by GST-NEMO and [.sup.35 S]-labeled NEMO by GST-IKK.beta.-(644-756). To test the ability of the NBD peptides to enter cells and inhibit the IKK.beta.-NEMO interaction, HeLa cells were incubated with the peptides for different time

periods and immunoprecipitated the **IKK complex** using anti-NEMO. In agreement with the in vitro data (FIG. 5A), wild-type but not mutant disrupted the formation of the endogenous **IKK complex** (FIG. 5B).

Detail Description Paragraph - DETX (177):

[0194] The importance of the present invention can be viewed on two levels. First, Applicants have identified the structural requirements for the association of NEMO with the **IKKs** and found that association with IKK.beta. is dependent on three amino acids (D738, W739 and W741) within the NBD. Furthermore, NEMO not only functions in the activation of IKK.beta. but it also has a critical role in suppressing the intrinsic, basal activity of the **IKK complex**. The second level of importance is the obvious clinical use for drugs targeting the NBD. Applicants have demonstrated that a cell-permeable peptide encompassing the NBD is able to not only inhibit TNF.alpha.-induced NF-.kappa.B activation but also reduce expression of E-selectin, an NF-.kappa.B-dependent target gene, in primary human endothelial cells. The NBD is only six amino acids long, and therefore it is within the ability of one skilled in the art to design peptido-mimetic compounds that disrupt the core **IKK complex**. Since the effect of disrupting the **complex** is to increase the basal activity of the **IKK,** treatment with an NBD-targeting compound can avoid issues of toxicity, e.g., due to hepatocyte apoptosis, that might arise from administering drugs that completely abolish the activity of NF-.kappa.B. Hence, identification of the NBD is a means for the development of novel anti-inflammatory drugs that prevent activating signals from reaching the **IKK complex,** yet maintain a low level of NF-.kappa.B activity and avoid potential toxic side-effects.

Detail Description Paragraph - DETX (189):

[0202] This data demonstrates that disruption of the core **IKK complex** by a cell permeable NBD peptide that inhibits NF-.kappa.B activation prevents RANKL-induced osteoclast differentiation indicating that drugs specifically targeting the NBD will be effective for the treatment of osteoporosis. As an extension of these in vitro studies, the same peptides can be analyzed for their effects on osteoporosis in vivo. Ovarectomized mice (Charles River Labs) that exhibit severe osteoporosis are treated with the NBD peptides and the effects on bone density over a timecourse of treatment determined.

Detail Description Paragraph - DETX (197):

[0206] In contrast to the lack of effects of the mutations described above on either NEMO binding or NF-.kappa.B activation, alanine substitution of the aspartic acid residue within the NBD (D738) prevented IKK.beta. from associating with NEMO. Furthermore, this substitution led to a 2- to 3-fold increase in the basal NF-.kappa.B-activating ability of IKK.beta.. These results demonstrate a role for NEMO association in maintaining the basal activity of the **IKK complex**. Interestingly, treatment of HeLa cells with the cell-permeable NBD peptide also led to a modest increase in basal NF-.kappa.B

activity further supporting the concept that loss of NEMO association leads to increased basal **IKK** activity.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030054450 A1

TITLE: Composition and method for reconstituting IkB kinase in yeast and methods of using same

PUBLICATION-DATE: March 20, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 079949

DATE FILED: February 19, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60269499 20010216 US

US-CL-CURRENT: 435/69.1, 435/194 , 435/254.2 , 435/320.1 , 435/34

ABSTRACT:

The invention provides a means for reconstituting I.kappa.B kinase in yeast in order to study the structure and regulation of **IKK** and to produce pharmacological therapies to block **IKK**. This invention can be used to express an **IKK complex** that is biochemically identical to **IKK** isolated from native cells and in coupled in vitro kinase assays to screen for its upstream regulators. The **IKK** expressed by reconstituting the yeast can be used to screen for unknown substrates and for pharmacological therapies that block its activity. The invention could also be used to screen for compounds that enhance its activity. The **IKK** may also be used as a source of material for crystallization and X-ray diffraction analysis.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/269,499, filed Feb. 16, 2001, the entire disclosure of which is hereby incorporated by reference in its entirety for all purposes.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

The invention provides a means for reconstituting I.kappa.B kinase in yeast in order to study the structure and regulation of **IKK** and to produce pharmacological therapies to block **IKK**. This invention can be used to express an **IKK complex** that is biochemically identical to **IKK** isolated from native cells and in coupled in vitro kinase assays to screen for its upstream regulators. The **IKK** expressed by reconstituting the yeast can be used to screen for unknown substrates and for pharmacological therapies that block its activity. The invention could also be used to screen for compounds that enhance its activity. The **IKK** may also be used as a source of material for crystallization and X-ray diffraction analysis.

Summary of Invention Paragraph - BSTX (9):

[0008] In resting cells, NF-.kappa.B is found predominantly in the cytoplasm in a **complex with IKK,** an inhibitory subunit, which sequesters NF-.kappa.B and prevents its migration to the nucleus (2). Diverse stimuli lead to phosphorylation of two serine residues, S32 and S36 on I.kappa.B.alpha., which targets I.kappa.B.alpha. for polyubiquitination and proteolytic degradation. This frees NF-.kappa.B to move to the nucleus where it binds with high affinity to .kappa.B elements in the promoter region of target genes (32).

Summary of Invention Paragraph - BSTX (11):

[0010] A diverse array of signals traverse multiple signaling pathways to stimulate NF-.kappa.B. All the signaling pathways, with the exception of ultraviolet radiation (15), converge at a specific critical regulatory point: the phosphorylation of amino terminal serines on I.kappa.B. This phosphorylation is catalyzed by a large kinase **complex,** I.kappa.B kinase ("**IKK**") (8, 20, 44). Because phosphorylation of I.kappa.B.alpha. by **IKK** is the key step in activation of NF-.kappa.B, understanding the structure and regulation of the **complex** is critical and, as discussed below, could be used to develop therapies to treat a variety of inflammatory and autoimmune diseases.

Summary of Invention Paragraph - BSTX (13):

[0012] Some aspects of the structure of **IKK** are known in the art (43). In 1997, a research group, including one of the inventors of the present invention, isolated and sequenced **IKK**. (See U.S. Pat. No. 6,242,253). **IKK** is composed of two homologous kinase subunits IKK.alpha. and IKK.beta. (85 kD and 87 kD respectively) and a 52 kD regulatory subunit IKK.gamma. (8, 42, 44). The .alpha. and .beta. subunits are associated with each other via their leucine zippers (42). It is believed that an .alpha.-helical regions towards the N-terminus of IKK.gamma. interacts with six amino acids at the C-terminus of IKK.alpha. and IKK.beta. (19). IKK.gamma. is required for activation of **IKK** in response to TNF and other stimuli (27). Experiments show that interrupting this interaction leads to a higher basal **IKK** activity but prevents stimulation of **IKK** by TNF.alpha. (19). Recombinant IKK.gamma. forms dimers and trimers (27), and it is possible that IKK.gamma. mediates formation of the large **IKK complex**.

Summary of Invention Paragraph - BSTX (14):

[0013] A number of molecules in the TNF.alpha. signaling pathway have been shown to be involved in activation of **IKK** by TNF-.alpha. and by IL-1. The binding of a ligand to a receptor induces receptor trimerization and subsequent recruitment of signaling proteins including TRADD, RIP, and TRAFs (which also trimerize). Forced oligomerization of these signaling proteins stimulates the downstream effects (3), suggesting that this is a key event in **IKK** activation. Experiments have indicated that the **IKK complex** (through IKK.gamma.) interacts with RIP and the TNF receptor after stimulation (45).

Summary of Invention Paragraph - BSTX (20):

[0019] Sf9 and mammalian systems also have the disadvantages of endogenous **IKK** and redundant factors that are not found with yeast. Studying signal transduction directly in mammalian or other higher eukaryotic cells is difficult because many signaling pathways have similar and redundant factors, and many of the signal transduction pathways intersect and act upon each other. Because **IKK** is a large **complex** composed of three different subunits, there may be multiple complexes of **IKK** which exist to respond to different signals. Furthermore, **IKK** responds to over 150 signals, studying **IKK** in mammalian cells is particularly difficult.

Summary of Invention Paragraph - BSTX (23):

[0022] As shown in U.S. Pat. No. 6,312,923, transducing yeast cells to express functional recombinant proteins is known in the art, the present invention was the first to succeed in reconstituting **IKK complex** in yeast. Because it was believed that yeast expressed a protein system analogous to mammalian **IKK,** those skilled in the art believed it would not be possible to reconstitute **IKK** in yeast. However, because the present inventors found that yeast does not express these proteins, they were able to develop disclosed methods and compositions.

Summary of Invention Paragraph - BSTX (25):

[0024] Furthermore, a biochemical approach to learning about **IKK** allows the a single variable at a time to be addressed. The inventors have discovered that yeast cells, S. cerevisiae in particular, lack TNF-.alpha. and NF-.kappa.B signaling pathways. Therefore, exogenously proteins expressed by the yeast cell do not affect the **IKK** signaling pathways. Because the only **IKK** expressed by the transformed yeast cell is from the plasmid insert, it is simple to test if a single molecule or subcellular fraction mutation affects the activity of the enzyme. **IKK** expressed in yeast can be used for clean mechanistic analysis. It can also be used to study the composition of the enzyme **complex** and how it is regulated.

Summary of Invention Paragraph - BSTX (26):

[0025] The heterologous expression of **IKK** allows the examination of the composition of the enzyme **complex** and how it is regulated. It allows for very clean mechanistic analysis that facilitates, for example, designing experiments on the function of specific residues or domains. In addition, by co-expressing putative upstream regulators, partial or entire signal transduction cascades can be reconstituted in a heterologous, in vivo, system without interference from endogenous molecules.

Summary of Invention Paragraph - BSTX (28):

[0026] The present invention provides a method for reconstituting **IKK** in yeast and the resulting composition of reconstituted **IKK complex**. Accordingly, the present invention provides a means to study the structure and regulation of **IKK** and to produce pharmacological therapies to block **IKK**.

Summary of Invention Paragraph - BSTX (30):

[0028] It is another object of the present invention to provide a composition of **IKK** reconstituted in yeast. In a related aspect, the invention is an **IKK complex** that is biochemically identical to **IKK** isolated from wild type cells.

Summary of Invention Paragraph - BSTX (31):

[0029] In yet another aspect of the invention, a mechanism for the regulation of the **IKK complex** is disclosed wherein IKK.gamma. regulates the autophosphorylation of the T loop residues in the kinases domain of IKK.beta.. When the T loop residues are phosphorylated, the kinase is active. This phosphorylation is required for activation of the **IKK complex**. In the inactive state, the T loop residues are not phosphorylated while the .gamma.BD serine(s) are phosphorylated, and the phosphorylation of the serine(s) prevents IKK.gamma. from facilitating self-activation. The activation of the **complex** requires dephosphorylation of these .gamma.BD serines, which then allows IKK.gamma. to facilitate autophosphorylation of IKK.beta. in the T loop.

Brief Description of Drawings Paragraph - DRTX (5):

[0035] FIG. 2. Human **IKK** Expressed in Yeast Forms a Large **Complex** Similar to **IKK** from HeLa Cells

Brief Description of Drawings Paragraph - DRTX (7):

[0037] Yeast expressing human IKK.alpha., IKK.beta., and IKK.gamma. were lysed, and the 65,000 g supernatant was applied to the superose 6 gel

filtration column and chromatographed. IKK.alpha..beta..gamma. activity eluted as a large **complex** (similar to **IKK** from HeLa cells). **IKK** activity from .alpha..beta..gamma.-expressing yeast was also seen in smaller complexes; most likely these complexes contain the catalytic subunits as dimers without IKK.gamma.. The activity from yeast expressing IKK.beta. only elutes as a small 158-230 kD **complex**.

Brief Description of Drawings Paragraph - DRTX (16):

[0046] However, as shown in lane 8, reconstitution with IKK.gamma. does not allow IKK.beta..gamma.BDEE to become activated. This result indicates that the analog of phosphorylated serines in the gamma binding domain prevents IKK.gamma. from facilitating self-activation of **IKK** and suggests that phosphorylation of these amino acids is a mechanism to maintain **IKK** in an inactive state. In lane 9, IKK.beta. with the 6 amino acids in the .gamma.BD domain at the C terminus (LDWSWL) were deleted and subsequently had a low level of activity, similar to wild-type IKK.beta.. But, as seen in lane 10, IKK.gamma. could not allow the **complex** to self-activate. This result indicates that the .gamma.BD is required for self-activation of the **complex**.

Detail Description Paragraph - DETX (3):

[0052] As used herein, the term "isolated," when used in reference to an IKB kinase **complex or to an IKK** subunit of the invention, means that the protein **complex** or subunit is relatively free from contaminating lipids, proteins, nucleic acids or other cellular material normally associated with an **IKK** in a cell.

Detail Description Paragraph - DETX (6):

[0055] The disclosed method for reconstituting **IKK** kinase involves the coexpression in yeast cells of genes encoding all three subunits of the **IKK** kinase **complex,** IKK.alpha., IKK.beta. and IKK.gamma.. The subunits can be introduced in the yeast strain on a stable plasmid (e.g. pESC), or it can be integrated into the yeast chromosome using standard techniques (46).

Detail Description Paragraph - DETX (7):

[0056] The choice of expression vectors for use in connection with the **IKK** subunits is not limited to a particular vector. Any expression vector suitable for use in yeast cells, including small yeast chromosomes ("YACs") and cosmids, is appropriate. The discussion related to experiments in the Examples section below describes particular vector, promoter and tag combinations that yielded meaningful results. However, many options are available for genetic markers, promoters and ancillary expression sequences. As discussed in greater detail below, the use of an inducible promoter to drive expression of the cDNA library is a preferred feature which provides a convenient means for demonstrating that observed changes in **IKK** activity are, in fact, dependent on the cDNA library

derived from the **IKK complex** subunits. Likewise, the inclusion of a tag, or a small polypeptide, facilitates determining the ratio of alpha to beta to gamma subunits were expressed by the transposed yeast cells.

Detail Description Paragraph - DETX (13):

[0062] The method of the present invention is not limited to the reconstitution of **IKK complex** in yeast. Rather, the method can be modified for use reconstituting a number of different kinases and proteins--especially those composed of multiple subunits.

Detail Description Paragraph - DETX (20):

[0068] For harvesting and lysing the yeast, all steps are performed at 4.degree. C. unless otherwise indicated. The yeast are first washed in 400 mM (NH.sub.4).sub.2SO.sub.4, 200 mM Tris-HCl, (pH 8.0), 10 mM MgCl.sub.2, 10% glycerol containing yeast protease inhibitors (2.5 .mu.g/ml leupeptin, 20 .mu.g/ml aprotinin, 2.5 .mu.g/ml antipain, 2 .mu.g/ml pepstatin, 1 mM PMSF, 0.1 .mu.g/ml chymostatin, and 1.1 .mu.g/ml phosphoramidon). 1-2 g of yeast pellet are resuspended in 2 mL lysis buffer (20 mM Tris (pH 7.6), 20 mM NaF, 20 mM .beta.-glycerophosphate, 0.5 mM Na.sub.3VO.sub.4, 2.5 mM sodium metabisuphite, 5 mM benzamidine, 1 mM EDTA, 0.5 mM EGTA, 10% glycerol, 300 mM NaCl, 1% triton X-100 with yeast protease inhibitors) in a capped 15 mL conical tube, frozen at -80.degree. C., and thawed on ice. Acid-washed 425-600.mu. glass beads (equal in volume to the yeast pellet) are added to the yeast, and the suspension is vortexed for 3.times.1 min (with 1 min incubation on ice between mixings). The suspension is then centrifuged at 3000g for 3 min, and the supernatant is collected. To extract more protein, 1 ml additional lysis buffer is added to the yeast, and the vortexing and centrifuigation and resuspension procedure is repeated an additional 8 times. To remove particulate material, the crude supernatant is centrifuged at 65,000 g for 1.5 hr, and the supernatant is collected and stored at -80.degree. C. For some experiments, **IKK** is partially purified using superose 6 gel filtration chromatography as described (44). The disclosed invention expresses an **IKK complex** that appears biochemically identical to **IKK** isolated from wild type cells. FIG. 1 shows a Western blot of yeast strains expressing HA-IKK.alpha., HA-IKK.beta., HA-IKK.beta..sub.KA (dominant negative) and HA-IKK.gamma. alone and in combination. The subunits for **IKK** were seen only in strains transformed with these genes.

Detail Description Paragraph - DETX (21):

[0069] **IKK** produced in yeast (yhIKK) forms a large **complex** like **IKK** from HeLa cell lysates as evidenced by gel filtration chromatography. As shown in FIG. 2, both **IKK** activity from HeLa cells and **IKK** activity from yeast expressing .alpha., .beta., and .gamma. elute from a superose 6 gel filtration column as a large .about.900 kD **complex**. This result indicates that the yhIKK.alpha..beta..gamma. we have expressed is native and that, most likely, the 900 kD **complex** contains no additional proteins. When only the catalytic

subunit is overexpressed in mammalian cells, IKK.gamma. is limiting and the catalytic subunit (without the IKK.gamma. subunit) elutes as an apparent dimer at 158-230 kD. A 158-230 kD **complex** is also seen in the yeast expressing .alpha., .beta., and .gamma., and this is probably due to an excess of catalytic subunits over IKK.gamma.; **IKK** activity from yeast expressing IKK.beta. only also elutes at 158-230 kD.

Detail Description Paragraph - DETX (24):

[0071] This example provides a method for identifying mechanisms for the regulation of the **IKK complex**. For example, by mutating to alanines (to prevent any phosphorylation) and/or to glutamic acids (to mimic the charge in the phosphorylated state) regulatory serines (177 and 181) in the kinase domain (T loop) and putative regulatory serines (740 and 750) in the gamma binding domain (.gamma.BD) of IKK.beta. and then expressing and partially purifying these mutated forms of IKK.beta. in yeast with and without IKK.gamma., the following mechanism for the regulation of the **IKK complex** is suggested: IKK.gamma. regulates the autophosphorylation of the T loop residues in the kinases domain of IKK.beta.. This phosphorylation is required for activation of the **IKK complex**. In the inactive state, the T loop residues are not phosphorylated while the .gamma.BD serine(s) are phosphorylated, and the phosphorylation of the serine(s) prevents IKK.gamma. from facilitating self-activation. The activation of the **complex** requires dephosphorylation of these .gamma.BD serines, which then allows IKK.gamma. to facilitate autophosphorylation of IKK.beta. in the T loop. When the T loop residues are phosphorylated, the kinase is active.

Detail Description Paragraph - DETX (32):

[0078] In another embodiment, the present invention can be used to develop a method for assaying **IKK** activity in situ in yeast. The present invention provides two methods for using the present invention to screen for upstream regulators of **IKK**. First, because the **IKK complex** reconstituted in yeast is only partially active, the protein extracts from cytokine stimulated mammalian cells can activate the **IKK complex** in coupled in vitro kinase assays. Such activity can be then purified biochemically to identify the protein components of it. Second, as described below in EXAMPLE IV, a system can be established to isolate potential negative regulators in situ in yeast. The in situ system is based on determining the activity of **IKK** in yeast by assessing an antibody that recognizes only the phosphorylated form of I-.kappa.B.alpha.ser 32.

Detail Description Paragraph - DETX (34):

[0080] As indicated by these assays, I.kappa.B.alpha. is phosphorylated in yeast by IKK.beta.:IKK.gamma. **complex** and the phosphorylation can be detected by phosphoantibodies to I.kappa.B.alpha.. I.kappa.B.alpha. was not phosphorylated in yeast that does not express **IKK complex** or expresses kinase defective IKK.beta.:IKK.gamma..

Detail Description Paragraph - DETX (45):

[0089] The invented composition can also be used to screen for pharmacological therapies to block its activity. This can be accomplished in two ways: first, libraries of small molecule compounds can be tested in in vitro kinase assays to inhibit or further activate the **IKK complex** made in yeast. For example, partially purified **IKK complex** from yeast can be incubated with a small molecule prior to testing its activity by in vitro kinase assay. Because this is a simple assay, a large number of compounds can be tested to determine if they inhibit or activate **IKK**. Second, the yeast system described above in EXAMPLE III, can be used to screen for small molecules that would inhibit or further activate the activity of **IKK** in situ (yeast).

Detail Description Paragraph - DETX (47):

[0090] In another embodiment, the present invention can be used as a source of material for crystallization and X-ray diffraction analysis. The present invention can be used to produce and purify highly homogenous **IKK** complexes of various compositions in large quantities. Yeast expressing **IKK** of different compositions can be grown in large quantities (e.g. 10 to 20 liters) and lysed. **IKK** complexes can be purified by affinity chromatography. This material can be used for producing crystals for X-ray differentiation and structural analysis. Purified **IKK** can also be used for cryo-EM single particle reconstitution analysis of **IKK complex** structure at lower resolutions. Because this method requires small amounts of purified proteins, the yeast system disclosed in the present invention can be used to produce various mutant forms of **IKK** subunits for structure determination. Comparison of wild type and mutant structures of **IKK** provides information for rational and targeted design of inhibitor and activators for the **complex**.

Detail Description Paragraph - DETX (77):

[0119] 27. Rothwarf, D. M., E. Zandi, G. Natoli, and M. Karin 1998. **IKK**-gamma is an essential regulatory subunit of the IkB kinase **complex**. Nature. 395:297-300.

Detail Description Paragraph - DETX (94):

[0136] 44. Zandi, E., D. M. Rothwarf, M. Delhase, M. Hayakawa, and M. Karin 1997. The IkappaB kinase **complex (IKK)** contains two kinase subunits, IKKalpha and IKKbeta, necessary for IkappaB phosphorylation and NF-kappaB activation. Cell. 91:243-252.

Claims Text - CLTX (25):

24. A heterologously expressed **IKK complex, wherein said IKK** is expressed by yeast.

Claims Text - CLTX (26):

25. The composition of claim 24, wherein said **IKK complex** is comprised of IKK.alpha., IKK.beta., and IKK.gamma. subunits.

Claims Text - CLTX (27):

26. The composition of claim 24, wherein said **IKK complex** is produced by the method of claim 1.

Claims Text - CLTX (28):

27. A heterologously expressed **IKK complex,** wherein said IKK.gamma. protein subunit regulates phosphorylation of serine residues in the activation of T loop kinase domain of **IKK** catalytic subunits.

Claims Text - CLTX (29):

28. The method of claim 27, wherein said **IKK complex** is activated by the dephosphorylation of .gamma.BD serines.

Claims Text - CLTX (33):

32. A method for identifying upstream regulators of **IKK complex,** comprising the steps of: a. mutating the genes of one or more said **IKK** subunits; b. subcloning genes for **IKK** subunits into yeast expression vectors; c. transforming said yeast expression vectors into yeast; d. growing said yeast in a selective liquid media; e. controllably inducing the expression of said **IKK** subunits by means of inducible promoters; f. lysing said yeast; g. extracting said **IKK** protein; h. purifying said **IKK** protein; and i. comparing kinase activity of said **IKK** protein with wild type **IKK**.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030050270 A1

TITLE: Antisense modulation of Inhibitor-kappa B Kinase-beta expression

PUBLICATION-DATE: March 13, 2003

INVENTOR-INFORMATION:

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DATE FILED: May 24, 2002

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child PCT/US99/16959 19990728 WO

parent continuation-of 09197008 19981120 US GRANTED

parent-patent 5977341 US

US-CL-CURRENT: 514/44, 514/81 , 536/23.2

ABSTRACT:

Antisense compounds, compositions and methods are provided for modulating the expression of Inhibitor-kappa B Kinase-beta. The compositions comprise antisense compounds, particularly antisense oligonucleotides, targeted to nucleic acids encoding Inhibitor-kappa B Kinase-beta. Methods of using these compounds for modulation of Inhibitor-kappa B Kinase-beta expression and for treatment of diseases associated with expression of Inhibitor-kappa B Kinase-beta are provided.

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 09/856,246 filed Aug. 30, 2001, which is the national phase filing

of PCT application PCT/US99/16959, filed Jul. 28 1999, which is the PCT application of U.S. patent application Ser. No. 09/197,008, filed Nov. 20, 1998, now issued as U.S. Pat. No. 5,977,341.

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Detail Description Paragraph - DETX (189):

[0276] In a separate Inhibitor-kappa B Kinase-beta activity assay the Inhibitor kappa B Kinase-alpha/beta (**IKK**-alpha/beta) **complex** was immmunoprecipitated, stimulated by TNF-alpha as described above, and assayed for kinase activity using .sup.32P-labeled ATP with IKB-alpha as the substrate. Phosphorylated products were detected via autoradiography. When the **IKK**-alpha/beta **complex** was treated with 200 nM ISIS 100004, levels of phosphorylated IKB-alpha were 60% lower than the levels observed for treatment with ISIS 100013 (scrambled control oligonucleotide) or with no oligonucleotide treatment.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030045515 A1

TITLE: Combination medicament for treatment of neoplastic diseases

PUBLICATION-DATE: March 6, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 151094

DATE FILED: May 21, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60292928 20010524 US

US-CL-CURRENT: 514/210.2, 514/217.04 , 514/353

ABSTRACT:

Pharmaceutical compositions comprising, as a first anti-neoplastic drug, cyanoguanidine IKK inhibitors, and in particular compounds of formula I 1 wherein
n is 0, 1 or 2;
each R independently represents halogen, trifluoromethyl, hydroxy, C.sub.1-4 alkyl, C.sub.1-4 alkoxy, C.sub.1-4 alkoxycarbonyl, nitro, sulfo, cyano, amino or carboxy groups; Q is a straight or branched, saturated or unsaturated C.sub.4-20 divalent hydrocarbon radical;
X is a bond, amino, O, S, carbonyl, carbonylamino, aminocarbonyl, oxycarbonyloxy, oxycarbonyl, carbonyloxy, aminocarbonyloxy, aminothiocarbonyloxy, oxycarbonylamino or oxythiocarbonylamino;
A is di-(C.sub.1-4 alkoxy)phospinoyloxy, C.sub.1-4 alkoxycarbonyl, C.sub.1-4 alkoxycarbonylamino, C.sub.3-12 carbocyclic ring or C.sub.3-12 heterocarbocyclic ring optionally substituted with one or more R.sub.1; R.sub.1 being independently selected from the group consisting of halogen, trifluoromethyl, hydroxy, C.sub.1-4 alkyl, C.sub.1-4 alkoxy, C.sub.1-4 alkoxycarbonyl, nitro, cyano, amino, sulfo, carboxy, carboxamido, sulfamoyl or C.sub.1-4 hydroxyalkyl; in combination with a second anti-neoplastic drug are provided.

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Summary of Invention Paragraph - BSTX (13):

[0011] At the cellular level it is well recognised that Nuclear Factor .kappa.B (NF.kappa.B) plays a pivotal role in apoptosis and resistance to apoptosis. It is also described that an NF.kappa.B inhibitor, I.kappa.B, and an I.kappa.B kinase **complex, IKK,** control the level of activated NF.kappa.B [Levkau, 1, 227-233, 1999; Wang, Science, 274, 784-787, 196; Madrid, Molecular and Cellular Biology, 5, 1626-1638, 2000]. Accordingly, the NF.kappa.B-I.kappa.B-**IKK** system has been suggested as a target in the treatment of neoplastic diseases.

Summary of Invention Paragraph - BSTX (18):

[0015] It has surprisingly been found that certain cyanoguanidine compounds are capable of modulating the level of activated NF.kappa.B through inhibition of the I.kappa.B kinase **complex** (abbreviated **IKK** in the following), thereby preventing resistance to the apoptosis effected by other anti-neoplastic drugs and ionising radiation. Cyanoguanidine compounds are thus able to increase the effect of other anti-neoplastic treatments. Synergistic effects may therefore be obtained in the treatment of patients with neoplastic diseases by combining treatment with cyanoguanidine compounds with other types of anti-neoplastic treatment, e.g. treatment with chemotherapeutic agents, hormonal agents, biological response modifiers, angiogenesis inhibitors, differentiating agents and ionising radiation.

Summary of Invention Paragraph - BSTX (67):

[0063] NF.kappa.B is a member of the Rel family of transcription factors, which are ubiquitous in animal cells. Rel proteins can form dimers, the most common of which is designated NF.kappa.B. NF.kappa.B is a p50/p65 heterodimer which can activate transcription of genes containing the appropriate .kappa.B binding site. In non-stimulated cells, NF.kappa.B is maintained in the cytoplasm by an interaction with NF.kappa.B inhibiting proteins, the I.kappa.BS. In response to cell stimulation by e.g. anti-neoplastic drugs or ionising radiation an I.kappa.B kinase **complex (IKK**) is rapidly activated and phosporylates two serine residues in the NF.kappa.B binding domain of I.kappa.B. The phophorylated I.kappa.B is then degraded by a 26S proteasome whereas NF.kappa.B is spared from degradation and translocates into the nucleus [Wang, Science, 274, 784-787, 1996, Cusak, Cancer Research, 60, 2323-2330, 2000; Karin, Immunology, 12, 2000, 85-98]. NF.kappa.B is thus always present in the cell, but in an inactivated form in non-stimulated cells. After translocation into the nucleus NF.kappa.B induces inter alia the anti-apoptotic genes c-IAP1, c-IAP2, TRAF1, TRAF2, Bfl-1/A1, Bcl-X.sub.L and Mn-SOD [Platel, Oncogene, 19, 2000, 4159-4169], which bring about resistance in the cells to apoptosis. This effect is referred to as the anti-apoptotic effect of NF.kappa.B, and the effect may be quantified by measuring the expression of compounds encoded by any of said genes, by any suitable means known in the art, in the presence and absence of compounds modulating the level of activated

NK.kappa.B. Any compound capable of reducing the transcription of said genes to a level less than 50%, e.g. less than 30%, such as less than 20% of the level in the absence of said compound is said to reduce the antiapoptotic effect of NK.kappa.B. Anti-neoplastic drugs and ionising radiation thus induce resistance in the cells to the treatments, which render them ineffective. Accordingly, activated NF.kappa.B is a key factor in induced resistance in e.g. cancer cells to anti-neoplastic drugs and/or to ionising radiation. This is further supported by the fact that constitutively activated NF.kappa.B is found in cells from resistant cancer tumours [Patel, Oncogene, 19, 4159-4169, 2000].

Summary of Invention Paragraph - BSTX (71):

[0067] The I.kappa.B kinase **complex** consist of three subunits, namely IKK.alpha., IKK.beta. and IKK.gamma., with a combined molecular weight of 900 kDa. IKK.alpha. and IKK.beta. both exhibit I.kappa.B kinase activity and phophorylate I.kappa.B, whereas IKK.gamma. is a regulatory subunit. IKK.alpha. is 85 kDa protein and IKK.beta. is a 87 kDa protein, and the two subunits show a large degree of homology. Whereas both IKK.alpha. and IKK.beta. are catalytically active, it has surprisingly been shown that only IKK.beta. is essential for **IKK** phosphorylation of I.kappa.B.

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TITLE: Methods for treatment of insulin resistance

PUBLICATION-DATE: March 6, 2003

INVENTOR-INFORMATION:

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parent continuation-in-part-of 09636150 20000810 US GRANTED

parent-patent 6468755 US

US-CL-CURRENT: 435/7.2, 435/15 , 435/7.21

ABSTRACT:

The invention features a method of identifying, evaluating or making a compound or agent, e.g., a candidate compound or agent, for treatment of a disorder characterized by insulin resistance. The method includes evaluating the ability of a compound or agent to bind IKK-.beta. or modulate IKK-.beta. activity, to thereby identify a compound or agent for the treatment of a disorder characterized by insulin resistance. The invention also features compounds for treating insulin resistance identified by such methods, and methods of treating a subject having a disorder characterized by insulin resistance by administering such agents.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part application of and claims priority to U.S. application Ser. No. 09/636,150, filed on Aug. 10, 2000, and U.S. Provisional Application Serial No. 60/148,037, filed Aug. 10, 1999, the contents of which are incorporated herein by reference.

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Summary of Invention Paragraph - BSTX (14):

[0013] In a preferred embodiment, the ability of a test compound to bind **IKK**-.beta. can be determined by detecting the formation of a **complex** between **IKK**-.beta. and the compound. The presence of the compound in **complex** indicates the ability to bind **IKK**-.beta..

Summary of Invention Paragraph - BSTX (25):

[0024] In a preferred embodiment, the ability of a test compound to bind **IKK**-.beta. can be determined by detecting the formation of a **complex** between **IKK**-.beta. and the compound. The presence of the compound in **complex** indicates the ability to bind **IKK**-.beta..

Detail Description Paragraph - DETX (31):

[0081] Culture cells were used as follows to investigate the mechanisms relating to salicylate treatment to the in vivo reversal of insulin resistance. TNF-.alpha. treatment of 3T3-L1 adipocytes induced `insulin resistance`, as judged by significant decreases in insulin-stimulated tyrosine phosphorylation of IR .beta.-subunit (42.+-.11%) and IRS-1 (37.+-.9%). TNF-.alpha. mediated `insulin resistance` was reversed by pretreatment with high-dose (5 mM) aspirin. IR and IRS-1 phosphorylation levels were restored to 126.+-.24% and 136.+-.35%, respectively, compared to untreated controls; IR and IRS-1 protein levels were unchanged in TNF-.alpha. and aspirin-treated cells. TNF-.alpha. activates a cascade of adapters and kinases, including TRADD, RIP, TRAF2, and TAB1, which act upstream of JNK, p38 MAPK, and the **IKK complex**. Okadaic acid and calyculin A, two phosphatase inhibitors, also activate IKK.beta. (DiDonato et al. (1997) Nature 388:548; Harhaj & Sun (1997) J Biol Chem 272:5409), but without activating upstream elements in the TNF-.alpha. signaling cascade. Okadaic acid and calyculin A also induce `insulin resistance` in isolated tissues and cultured cells (Robinson et al (1993) Am J Physiol 265:E36; Paz et al (1997) J. Biol Chem 272:29911). Therefore, it was determined whether aspirin would reverse `insulin resistance` caused by these inhibitors. Marked reductions in insulin-stimulated IR (29.+-.12%) and IRS-1 (16.+-.2%) tyrosine-phosphorylation were prevented by incubating the cells with high-dose aspirin (109.+-.15% and 93.+-.25%, respectively). Notably, the reduced electrophoretic mobility of IRS-1 due to calyculin A-induced phosphorylation was reversed with aspirin, further suggesting that aspirin's ability to reverse insulin resistance might occur through reduced Ser/Thr phosphorylation of components in the insulin signaling cascade.

Detail Description Paragraph - DETX (35):

[0085] TNF-.alpha. does not appear to contribute to insulin resistance in type 2 diabetes and syndrome X, as biological blockers of TNF-.alpha. do not

alter insulin sensitivity (Ofei et al. (1996) Diabetes 45:881; Paquot et al. (2000) J Clin Endocrinol Metab 85:1316). However, TNF-.alpha. is a potential mediator of acquired insulin resistance (Lang et al. (1992) Endocrinology 130:43; Feinstein et al. (1993) J Biol Chem 268:26055; Hotamisligil et al. (1993) Science 259:87; Hotamisligil et al. (1994) J Clin Invest 94:1543). TNF-.alpha. activates the **IKK complex**. TNF-.alpha. treatment of untransfected 293 cells reduced insulin-stimulated IR activation to 29.+-.2% of untreated controls. Expression of kinase deficient, dominant inhibitory IKK.alpha.(K44A) or IKK.beta.(K44A) reversed TNF-.alpha.-inhibited IR activation. In fact, dominant-inhibitory IKK.beta. caused a 60% increase in insulin-stimulated IR tyrosine-phosphorylation over controls, whether or not cells had been treated with TNF-.alpha.. Similar effects were seen with AKT. TNF-.alpha. treatment reduced AKT activation (18.+-.15%), and this was reversed by IKK.beta.(K44A) expression (174.+-.38%). Active **IKK** kinases thus mediate `insulin resistance` in cultured cells, and the inactive kinases act as dominant inhibitors to block TNF-.alpha.0 induced insulin resistance. The consistent ability of dominant-inhibitory IKK.beta. to elevate IR signaling well above the normal level indicates that **IKK** inhibits insulin signaling even in the absence of TNF-.alpha.. There is in vivo support for this, as well. Fa/+ rats and ob/+ mice (see FIG. 1) and Sprague-Dawley rats that are not insulin resistant, obese, or diabetic, show increased insulin sensitivity in response to aspirin treatment.

Claims Text - CLTX (8):

7. The method of claim 2, wherein the ability of the compound to bind **IKK**-.beta. is determined by detecting the formation of a **complex** between **IKK**-.beta. and the compound.

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PGPUB-FILING-TYPE: new

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TITLE: Novel protein TAB2

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INVENTOR-INFORMATION:

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parent continuation-in-part-of PCT/JP99/06466 19991119 US UNKNOWN

US-CL-CURRENT: 435/69.1, 536/23.1

ABSTRACT:

A novel signal transducer TAB2 which acts as an adapter molecule of TRAF6 and TAK1 and mediates the activation of TAK1 in the signal transduction of IL-1 was isolated. TAB2 induced the activation of NF-.kappa.B and JNK by IL-1. The signal transduction by IL-1 was inhibited by inhibiting the signal transduction of TAB2 with the use of a dominant negative mutant of TAB2. A compound inhibiting the signal transduction in TAB2 is useful as an anti-inflammatory drug.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (5):

[0003] Recent studies have provided a model for how the IL-1 signal transduction cascade is regulated. The first signaling event for IL-1 is a ligand-induced **complex** formation of the type I receptor (IL-1RI) and the receptor accessory protein (IL-1RAcP) (Greenfeder et al., J. Biol. Chem. 270:13757-13765, 1995; Huang et al., Proc. Natl. Acad. Sci. USA 94:12829-12832, 1997; Korherr et al., Eur. J. Immunol. 27:262-267, 1997; Wesche et al., J. Biol. Chem. 272:7727-7731, 1997). The cytosolic myeloid differentiation protein MyD88 is next recruited to this **complex** (Cao et al., Science 271:1128-1131, 1996; Muzio et al., Science 278:1612-1615, 1997; Wesche

et al., Immunity 7:837-847, 1997; Bums et al., J. Biol. Chem. 273:12203-12209, 1998), which in turn enables the association of the serine/threonine IL-1 receptor-associated kinase (IRAK). IRAK becomes highly phosphorylated, leaves the receptor **complex,** and interacts with TRAF6 (TNF receptor-associated factor 6), which is required for IL-1-induced JNK and NF-.kappa.B activation (Cao et al., Nature 383:443-446, 1996; Yamin et al., J. Biol. Chem. 272:21540-21547, 1997; Lomaga et al., Genes Dev. 13:1015-1024, 1999). Another serine/threonine kinase, NF-.kappa.B -inducing kinase (NIK), is believed to be a downstream component in activating NF-.kappa.B, but not in the JNK activation, in response to IL-1 (Malinin et al., Nature 385:540-544, 1997). Recently, two I.kappa.B kinases (IKK.alpha./**IKK1** and IKK.beta./**IKK2**) have been implicated in signal-induced phosphorylation of the I.kappa.B proteins (DiDonato et al., Nature 388:548-554, 1997; Mercurio et al., Science 278:860-866, 1997; Regnier et al., Cell 90:373-383, 1997; Woronicz et al., Science 278:866-869, 1997; Zandi et al., Cell 91:243-252, 1997). The **IKKs** are components of a large **complex,** which contain NEMO (NF-.kappa.B essential modulator)/IKK.gamma. (Rothwarf et al., Nature 395:297-300, 1998; Yamaoka et al., Cell 93:1231-1240, 1998). The present inventors have recently demonstrated that the protein kinase TAK1 is involved in the IL-1 signaling pathway (Ninomiya-Tsuji et al., Nature 398:252-256, 1999). Following exposure of the cells to IL-1, endogenous TAK1 is recruited to the TRAF6 **complex,** where it becomes activated. Activated TAK1 then stimulates a MAP kinase cascade leading to JNK activation and a NIK-**IKK** cascade leading to NF-.kappa.B activation. Thus, TAK1 is positioned downstream of TRAF6 in the IL-1-activated signaling cascade. This suggests that the bifurcation of the IL-1-induced JNK and NF-.kappa.B activation pathways occurs at the level of TAK1.

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TITLE: Diagnosis and treatment of medical conditions associated with defective NFkappa B(NF-kappaB) activation

PUBLICATION-DATE: February 13, 2003

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APPL-NO: 09/ 863049

DATE FILED: May 22, 2001

RELATED-US-APPL-DATA:

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US-CL-CURRENT: 435/7.1

ABSTRACT:

Incontinentia Pigmenti (IP) is a neurocutaneous genodermatosis that segregates as an X-linked dominant disorder with a high probability of prenatal male lethality. A locus in Xq28 containing NF-.kappa.B Essential Modulator, a gene product involved in the activation of NF-kB and central to many pro-inflammatory and apoptotic pathways, contains mutations in the majority of cases of IP. Disclosed are methods, compositions and kits directed to a defect in a NF-.kappa.B related disease such as IP.

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 60/206,223, filed May 22, 2000.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (6):

[0006] Linkage of the IP locus to markers in distal Xq28 (DXS52-tel) was established with close linkage to the gene for factor VIII (Sefiani, 1989; Smahi, 1994; Parrish, 1996; Jouet, 1997) Many genes from this region have been excluded by extensive mutation screening (Heiss, 1999; Aradhya, 2000; Woffendin, 2000; Das, 1994). Recently, a gene intimately involved in inflammatory responses, NEMO/IKK.gamma., has been mapped 200 kb proximal to the factor VIII locus (Jin, 1999). NEMO is central to the activation of the ubiquitous transcription factor NF-.kappa.B (Yamaoka, 1998; Rudolph, 2000). The NF-.kappa.B/Rel family of transcription factors plays a particularly important role in inflammatory and immune responses, in cellular stress, and in regulating apoptosis (Ghosh, 1998; Baldwin, 1996). Their activity is induced by a variety of different stimuli including pro-inflammatory cytokines such as interleukin-1 (IL-1) and Tumour Necrosis Factor (TNF). The immediate responsiveness required of such a key regulator is effected by an unique mechanism whereby NF-.kappa.B homo- or heterodimers are sequestered in the cytoplasm through interaction with an inhibitory molecule of the IKB family (three different species exist in cells: I.kappa.B.alpha., I.kappa.B.beta. and I.kappa.B.epsilon.). Upon cytokine stimulation the I.kappa.B molecules are phosphorylated on two Ser residues, then polyubiquitinated and degraded through the ubiquitin-proteasome pathway. NF-.kappa.B is therefore free to translocate to the nucleus and to activate its target genes. This phosphorylation event is carried out by a high molecular weight, multiprotein kinase **complex** containing two subunits with kinase activity (**IKK1**/.alpha. and **IKK2**/.beta.) (Zandi, 1999; Mercurio, 1999). A third component of this **complex** is a 48 kDa protein with no apparent catalytic activity, called NEMO (NF-.kappa.B Essential Modulator), IKK.gamma., or **IKKAP** (Yamaoka, 1998; Rothwarf, 1998; Mercurio, 1999). NEMO directly interacts with the kinase subunits and is required for activation of the kinase **complex** in response to extracellular (or intracellular) stimuli: its absence results in a complete inhibition of NF-.kappa.B activation.

Detail Description Paragraph - DETX (25):

[0243] NEMO has been shown to play an essential role in the NF-.kappa.B activation process (Yamaoka, 1998). NF-.kappa.B homo- or heterodimers are sequestered in the cytoplasm through interaction with an inhibitory molecule of the IkB family. Upon cytokine stimulation, the IkB molecules are phosphorylated, polyubiquitinated and degraded through the ubiquitin-proteasome pathway (Ghosh et al., 1998; Rothwarf and Karin, 1999). NF-.kappa.B is then free to translocate to the nucleus and to activate its target genes. This phosphorylation event is carried out by a high molecular weight, multiprotein, kinase **complex** containing two subunits with kinase activity (**IKK1**/a and **IKK2**/b). The third known component of this **IKK complex** is NEMO (**Ikkg, IKKAP** or Human Gene Nomenclature name: IKBKG) a 48 kDa protein with no apparent catalytic activity that directly interacts with the kinase subunits and is for activation of the kinase **complex** in response to extracellular (or intracellular) stimuli: its absence results in a complete inhibition of NF-.kappa.B activation.

Detail Description Paragraph - DETX (36):

[0252] That NEMO activity is indeed ablated by the rearrangement has been shown for two cell lines expressing this mutation. Can the pathology observed in IP male and female patients therefore be explained in terms of NEMO function? NEMO is a structural/regulatory component of the **IKK complex** that also contains IKK.alpha./1 and IKK.beta./2 (Yamaoka, 1998; Courtois, 1997; Rothwarf, 1998; Mercurio, 1999). Ablation of NEMO activity results in the inability of a cell to activate NF-.kappa.B in response to a series of stimuli (Courtois, 1997; Yamaoka, 1998). The NF-.kappa.B signalling pathway itself has been implicated in immune, inflammatory, and apoptotic responses (for a review see Ghosh, 1998; Baldwin, 1996). Recently, the genes encoding the components of the **IKK complex,** including NEMO, have been inactivated by homologous recombination. Inactivation of **IKK2** (which does not entirely abolish NF-.kappa.B activation, probably due to partial compensation by the **IKK1** kinase subunit) resulted in embryonic death due to massive liver apoptosis at day 14 (Li, 1999; Li, 1999; Tanaka, 1999) while that of NEMO (which apparently results in a complete block in NF-.kappa.B activation) resulted in death of the male embryos at day 12 with a similar phenotype (murine NEMO is also located on the X-chromosome). Apparently the females were normal. A similarly dramatic phenotype was observed when the gene encoding re1A, the most ubiquitously expressed and most potent transcriptional activator of the NF-.kappa.B family, was inactivated: the mice died at embryonic day 15-16 from massive liver apoptosis (Beg, 1995). Interestingly this apoptosis is due to the pro-apoptotic effect of TNF, as demonstrated by the viability of mice carrying an inactivation of both the re1A and the TNF genes (Doi, 1999). This observation is in keeping with the high sensitivity to TNF-induced apoptosis in cell lines derived from IP patients (FIG. 5D). In both mice and humans, therefore, the complete absence (or lack of activity) of NEMO results in early lethality of the affected males. Post mortem examination of a few human affected males has suggested the involvement of an abnormal immune response, as expected from a defect in NF-.kappa.B signalling.

Detail Description Paragraph - DETX (39):

[0255] Another interesting hint comes from the analysis of mice carrying an inactivation of the **IKK1** component of the **complex** that includes NEMO (Hu, 1999; Li, 1999; Takeda, 1999). Inactivation of this kinase results in a completely unexpected phenotype: the resulting mice exhibit an almost intact activation of NF-.kappa.B by pro-inflammatory stimuli but show multiple defects in morphogenetic events, including limb and skeletal patterning and proliferation and differentiation of epidermal keratinocytes. Such an epidermal phenotype has also been observed with transgenic mice expressing a dominant negative version of I.kappa.Ba under skin-specific promoter control (Seitz, 2000; van Hogerlinden, 1999; Seitz, 1998). At the skin level NF-.kappa.B appears to play a dual role: it controls cell growth in the stratified epithelium and regulates apoptosis. Defect in both pathways may explain the characteristic skin lesions observed in IP2.

Detail Description Paragraph - DETX (156):

[0348] Isrel, A. The **IKK complex**: an integrator of all signals that activate NF-.kappa.B? Trends Cell Biol 10, 129-133 (2000).

Detail Description Paragraph - DETX (167):

[0359] Mercurio, F. et al. I.kappa.B kinase (**IKK**)-associated protein 1, a common component of the heterogeneous **IKK complex**. Mol Cell Biol 19, 1526-1538 (1999).

Detail Description Paragraph - DETX (174):

[0366] Rothwarf, D. M., Zandi, E., Natoli, G. & Karin, M. **IKK**-.gamma. is an essential regulatory subunit of the I.kappa.B kinase **complex**. Nature 395, 297-300 (1998).

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030022898 A1

TITLE: Methods of treating inflammatory and immune diseases using inhibitors of IkappaB kinase (IKK)

PUBLICATION-DATE: January 30, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 062847

DATE FILED: February 1, 2002

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US-CL-CURRENT: 514/250

ABSTRACT:

The present invention describes methods of preventing and treating inflammatory and immune-related diseases or disorders using inhibitors of I.kappa.B kinase (IKK). Also described are IKK inhibitors effective for the prevention and treatment of inflammatory and immune-related diseases or disorders, as demonstrated in vivo. Further embodiments of the present invention relate to a specific IKK inhibitors, 4(2'-aminoethyl)amino-1,8-- dimethylimidazo(1,2-a) quinoxaline and compounds of formula (I), salts thereof, and pharmaceutical compositions.

RELATED INVENTIONS

[0001] This application claims benefit to application U.S. Serial No. 60/223,304, filed Oct. 3, 2000 and application U.S. Serial No. 60/265,853, filed Feb. 1, 2001, and is a continuation-in-part of U.S. Ser. No.

09/965,977, filed Sep. 27, 2001. The contents of all of which are hereby incorporated by reference herein in their entireties.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (8):

[0007] Potential inhibitors of NF-.kappa.B and/or the NF-.kappa.B pathway have been identified as including Interleukin-10, glucocorticoids, salicylates, nitric oxide, and other immunosuppressants. I.kappa.B is a cytoplasmic protein that controls NF-.kappa.B activity by retaining NF-.kappa.B in the cytoplasm. I.kappa.B is phosphorylated by the I.kappa.B kinase (**IKK**), which has two isoforms, **IKK-1** (or I.kappa.B kinase .alpha., IKK.alpha.) and **IKK-2** (or I.kappa.B kinase .beta., IKK.beta.). Upon phosphorylation of I.kappa.B by **IKK,** NF-.kappa.B is rapidly released into the cell and translocates to the nucleus where it binds to the promoters of many genes and up-regulates the transcription of pro-inflammatory genes. Inhibitors of **IKK** can block the phosphorylation of I.kappa.B and further downstream effects, specifically those associated with NF-.kappa.B transcription factors. Glucocorticoids reportedly inhibit NF-.kappa.B activity by two mechanisms, i.e., upregulating I.kappa.B protein levels and inhibiting NF-.kappa.B subunits. Nitric oxide also reportedly inhibits NF-.kappa.B through upregulation of I.kappa.B. However, these mechanisms of interaction are **complex**; for example, production of nitric oxide in lymphocytes reportedly enhances NF-.kappa.B activity.

Detail Description Paragraph - DETX (151):

[0196] Assays for measuring the inhibitory potential of test compounds, specifically compound 6, (4(2'-aminoethyl)amino-1,8-dimethylimidazo(1,2-a-) quinoxaline), against **IKK** activity employed .sup.33P labeled ATP and a recombinant I.kappa.B-.alpha. as substrates. In this assay, test compound was added to a solution of 0.5 mM I.kappa.B-.alpha. in 40 mM Tris-HCl, pH 8, containing 4 mM MgCl.sub.2, 1 mM dithiothreitol, and 2 mM .sup.33P-labeled ATP. **IKK** enzyme (either the multisubunit **complex** from HeLa cells, recombinantly expressed **IKK-1,** or recombinantly expressed **IKK-2**) was then added to initiate the reaction. The multisubunit **complex** was isolated according to the procedure of Lee et al. [(1997) Cell 88:213-222] or Mercurio et al. [(1997) 278:860-866]. The recombinant **IKK-1 and IKK-2** were expressed using the procedure of Burke et al. [(1999) J. Biol. Chem. 274:36146-36152]. After incubating for 10 minutes at 30.degree. C., the reaction was quenched by the addition of EDTA to a final concentration of 30 mM. In order to separate the phosphorylated I.kappa.B-.alpha. (Santa Cruz Biotechnology; Santa Cruz, Calif.) product from unreacted ATP, sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed. Phosphoimager analysis of the amount of .sup.33P incorporation into I.kappa.B-.alpha. was executed using a Molecular Dynamics 445 Phosphoimager, and the inhibition of **IKK** activity by the test compound was calculated from control samples containing no inhibitor. The IC.sub.50 is defined as the concentration of test compound which produces 50% inhibition of **IKK** activity.

PGPUB-DOCUMENT-NUMBER: 20030017983

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030017983 A1

TITLE: Novel molecules of the pyrin/NBS/LRR protein family and uses thereof

PUBLICATION-DATE: January 23, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 124498

DATE FILED: April 17, 2002

RELATED-US-APPL-DATA:

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parent continuation-in-part-of 10066521 20020131 US PENDING

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non-provisional-of-provisional 60318645 20010910 US

US-CL-CURRENT: 514/12, 435/189 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

Novel PYRIN-2, PYRIN-3, PYRIN-5, PYRIN-6, PYRIN-7, PYRIN-8, PYRIN-10, and PYRIN-11 polypeptides, proteins, and nucleic acid molecules are disclosed. In addition to isolated PYRIN-2, PYRIN-3, PYRIN-5, PYRIN-6, PYRIN-7, PYRIN-8, PYRIN-10, and PYRIN-11 proteins, the invention further provides PYRIN-2, PYRIN-3, PYRIN-5, PYRIN-6, PYRIN-7, PYRIN-8, PYRIN-10, and PYRIN-11 fusion proteins, antigenic peptides and anti-PYRIN-2, -PYRIN-3, -PYRIN-5, -PYRIN-6, -PYRIN-7, -PYRIN-8, -PYRIN-10, and -PYRIN-11 antibodies. The invention also provides PYRIN-2, PYRIN-3, PYRIN-5, PYRIN-6, PYRIN-7, PYRIN-8, PYRIN-10, and PYRIN-11 nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced and non-human transgenic animals in which a PYRIN-2, PYRIN-3, PYRIN-5, PYRIN-6, PYRIN-7, PYRIN-8, PYRIN-10, or PYRIN-11 gene has been introduced or disrupted. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.

RELATED APPLICATIONS

[0001] This application is a continuation-in-part and claims priority to U.S. application Ser. No. 10/066,521, filed on Jan. 31, 2002, which claims priority to U.S. provisional application No. 60/265,231, filed on Jan. 31, 2001, and U.S. provisional application No. 60/318,645, filed on Sep. 10, 2001, the contents of which are incorporated herein by reference.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (13):

[0124] FIGS. 12A-12C are graphs depicting: (A) PYRIN-8 synergizing with CARD-5 (ASC) to induce NF-kB activation; (B) the importance of the N-terminal PYRIN domain of PYRIN-8 in synergizing with CARD-5 (ASC) to induce NF-kB activation; and (C) the activation of NF-kB activity by PYRIN-8 and CARD-5 through the **IKK complex**.

Detail Description Paragraph - DETX (71):

[0196] NF-kB signaling occurred through the **IKK complex,** as demonstrated by dominant-negative versions of **IKK**-.gamma. and **IKK-2** blocking the ability of PYRIN-8 and CARD-5 to synergistically activate NF-kB (FIG. 12C, lanes 3 and 4). These data demonstrate that PYRIN-8 functions as an activator of CARD-5 activity and is an upstream regulator of NF-kB signaling.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030004192 A1

TITLE: Method of modulating NF-kB activity

PUBLICATION-DATE: January 2, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 153800

DATE FILED: May 24, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60292927 20010524 US

US-CL-CURRENT: 514/353, 514/336

ABSTRACT:

A method of modulating the level of activated, NF-.kappa.B in cells by contacting cells with a cyanoguanidine compound of general formula I 1 wherein
n is 0, 1 or 2;
each R independently represents halogen, trifluoromethyl, hydroxy, C.sub.1-4 alkyl, C.sub.1-4 alkoxy, C.sub.1-4 alkoxycarbonyl, nitro, cyano, amino, sulfo or carboxy groups;
Q is a straight or branched, saturated or unsaturated C.sub.4-20 divalent hydrocarbon radical;
X Is a bond, O, S, amino, carbonyl, carbonylamino, aminocarbonyl, oxycarbonyloxy, oxycarbonyl, carbonyloxy, aminocarbonyloxy, aminothlocarbonyloxy, oxycarbonylamino or oxythiocarbonylamino;
A is di-(C.sub.1-4 alkoxy)phosphinoyloxy, C.sub.1-4 alkoxycarbonyl, C.sub.1-4 alkoxycarbonylamino, saturated or unsaturated C.sub.3-12 carbocyclic ring or C.sub.3-12 heterocarbocyclic ring optionally substituted with one or more R.sub.1; R.sub.1 being independently selected from the group consisting of halogen, trifluoromethyl, hydroxy, C.sub.1-4 alkyl, C.sub.1-4 alkoxy, C.sub.1-4 alkoxycarbonyl, nitro, cyano, amino, carboxy, sulfo, carboxamido, sulfamoyl or C.sub.1-4 hydroxyalkyl;
or a pharmaceutically acceptable salt, N-oxide or N-substituted prodrug thereof, in an amount effective to modulate the activity of IKK.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (2):

[0001] The present invention relates to methods of modulating the activity of NF-.kappa.B and to methods of inhibiting the I.kappa.B **complex (IKK)** using cyanoguanidine derivatives.

Summary of Invention Paragraph - BSTX (13):

[0011] At the cellular level it is well recognised that nuclear factor .kappa.B (NF-.kappa.B) plays a pivotal role in apoptosis. It is also described that an NF-.kappa.B inhibitor, I.kappa.B, and an I.kappa.B kinase **complex, IKK,** control the level of activated NF.kappa.B [Levkau, 1, 227-233, 1999; Wang, Science, 274, 784-787, 196; Madrid, Molecular and Cellular Biology, 5, 1626-1638, 2000]. Accordingly, the NF-.kappa.B-I.kappa.B**-IKK** system has been suggested as a target in the treatment of neoplastic diseases.

Summary of Invention Paragraph - BSTX (29):

[0026] In a still further aspect, the invention relates to a method of inhibiting the **IKK complex** by contacting cells with a compound of general formula I, as defined above, in an amount effective to inhibit **IKK**.

Detail Description Paragraph - DETX (29):

[0061] NF-.kappa.B is a member of the Rel family of transcription factors which are ubiquitous in animal cells. Rel proteins can form dimers, the most common of which is designated NF-.kappa.B, NF-.kappa.S is a p50/p65 heterodimer which can activate transcription of genes containing the appropriate .kappa.B binding site. In non-stimulated cells, NF-.kappa.B is maintained in the cytoplasm by interaction with NF-.kappa.B inhibiting proteins, the I.kappa.Bs. In response to cell stimulation by e.g. anti-neoplastic drugs or ionising radiation an I.kappa.B kinase **complex (IKK)** is rapidly activated and phosporylates two serine residues in the NF-.kappa.B binding domain of I.kappa.B. The phophorylated I.kappa.B is then degraded by a 26S proteasome whereas NF-.kappa.B is spared from degradation and translocates into the nucleus [Wang, Science, 274, 784-787, 1996, Cusak, Cancer Research, 60, 2323-2330, 2000; Karin, Immunology, 12, 2000, 85-98]. NF-.kappa.B is thus always present in the cell, but in an inactivated form in non-stimulated cells. After translocation into the nucleus NF-.kappa.B induces inter alia the anti-apoptotic genes c-IAP1, c-IAP2, TRAF1, TRAF2, Bfl-1/A1, Bcl-X.sub.L and Mn-SOD [Patel, Oncogene, 19, 2000, 4159-41699], which bring about resistance in the cells to apoptosis. This effect is referred to as the anti-apoptotic effect of NF-.kappa.B, and the effect may be quantified by measuring the expression of gene products encoded by any of said genes, by any suitable means known in the art, in the presence and absence of compounds modulating the level of activated NK-.kappa.B. Any compound capable of reducing the transcription of one or more of said genes to a level of less than about 50%, e.g. less than about 30%, such as less than about 20% of the level in the absence of said

compound is said to reduce the anti-apoptotic effect of NK-.kappa.B. Anti-neoplastic drugs and ionising radiation thus induce resistance in the cells to the treatments, which render them ineffective. Accordingly, activated NF-.kappa.B is a key factor in induced resistance in e.g. cancer cells to chemotherapeutic drugs and/or to ionising radiation, This is further supported by the fact that constitutively activated NF-.kappa.B is found in cells from resistant cancer tumours [Patel, Oncogene, 19, 4159-4169, 2000]. Regardless of reduced resistance to any treatment, a reduction of the level of activated NF-.kappa.B In the cell, e.g. by controlling the activity of **IKK,** will reduce the expression levels of genes encoding for anti-apoptotic factors, thereby inducing apoptosis in the cells [Schwartz, Surgical Oncology, 8, 1999, 143-153].

Detail Description Paragraph - DETX (32):

[0064] The I.kappa.B kinase **complex (IKK**) consist of three subunits, namely IKK.alpha., IKK.beta. and IKK.gamma., with a combined molecular weight of 900 kDa. IKK.alpha. and IKK.beta. both exhibit I.kappa.B kinase activity and phophorylate I.kappa.B, whereas IKK.gamma. is a regulatory subunit. IKK.alpha. is 85 kDa protein and IKK.beta. is a 87 kDa protein, and the two subunits show a large degree of homology. Whereas both IKK.alpha. and IKK.beta. are catalytically active, it has surprisingly been shown that only IKK.beta. is essential for **IKK** phosphorylation of I.kappa.B. It has been found by the present inventors that compounds of general formula I are effective as inhibitors of IKK.beta. in particular.

Detail Description Paragraph - DETX (99):

[0116] **IKK** activity: Upon cellular activation by extracellular stimuli, I.kappa.B proteins are phosphorylated by a large I.kappa.B kinase **complex**. An in vitro **IKK** activity assay was established to evaluate a possible effect of compound A on the **IKK** activity. The THP-1 cells were stimulated with 1 .mu.g/ml LPS for 12 min, and then the cells were lysed and immunoprecipitated by an **IKK** antibody. The purified **IKK** was then pretreated with various concentrations of compound A ranging from 10.sup.-11 to 10.sup.-5 M for 30 min. prior to the **IKK** activity assay (FIG. 4). A "chemical zero-point" was introduced by treating the LPS-activated **IKK** with the IKK.beta. inhibitor myricetin (20 .mu.M) (S. H. Tsai et al., supra) to overcome the problem with a prestimulated kinase. A clear dose-response was observed as illustrated by the decrease of GST-I.kappa.B.alpha. phosphorylation In the compound A-treated samples. Four independent experiments were performed and the results are summarised on the plot (FIG. 4). The IC.sub.50 values range from 0.9 nM to 70 nM with a mean IC.sub.50 value of 8 nM.

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TITLE: Novel molecules of the PYRIN domain protein family and uses thereof

PUBLICATION-DATE: December 26, 2002

INVENTOR-INFORMATION:

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DATE FILED: December 20, 2001

RELATED-US-APPL-DATA:

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child 09653901 20000901 US

parent continuation-in-part-of 09506067 20000217 US ABANDONED

US-CL-CURRENT: 435/7.92

ABSTRACT:

Diagnostic, screening and therapeutic methods utilizing NBS-1 and PYRIN-1 are disclosed. The methods take advantage of the interactions between NBS-1 or PYRIN-1 and various proteins involved in apoptotic and inflammatory signaling pathways. Also disclosed are methods for identifying modulators of ASC and NF-.kappa.B.

[0001] This application is a continuation-in-part and claims the benefit of priority under 35 U.S.C. 120 of U.S. application Ser. No. 09/964,955, filed on Sep. 26, 2001; and is also a continuation-in-part and claims the benefit of priority of U.S. application Ser. No. 09/653,901, filed on Sep. 1, 2000; which is a continuation-in-part of U.S. application Ser. No. 09/506,067, filed on Feb. 17, 2000. The disclosures of the prior applications are considered part of and incorporated by reference in the disclosure of this

application.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (17):

[0052] FIG. 16 depicts the results of an NF-kB activity assay showing that PYRIN-1 and ASC activate NF-kB through the **IKK complex**. In this study 293T cells were transfected with a plasmid expressing ASC (32 ng) and a plasmid expressing PYRIN-1 (500 ng) and either empty vector (500 ng) or a plasmid expressing dominant negative mutants of IKK.gamma. (IKK.gamma.-DN), or **IKK2** (**IKK2**-DN). Immunoblot analysis was performed to monitor expression of PYRIN-1 (upper panel) and ASC (middle panel). Relative luciferase activities were measured as described (lower panel).

Detail Description Paragraph - DETX (43):

[0097] NF-kB signaling occurred through the **IKK complex** because dominant-negative versions of **IKK**-.DELTA. and **IKK-2** blocked the ability of PYRIN-1 to synergistically activate NF-kB FIG. 26, lanes 3 and 4). To determine the role of individual domains in NF-kB signaling, the ability of the PYRIN-1 truncation mutants to activate NF-kB was investigated. The N-terminal PYRIN domain of PYRIN-1 was essential for NF-kB signaling, since deletion of this domain (PYRIN-1.DELTA.PYRIN) eliminated the synergistic induction of NF-kB activity (FIG. 15, lane 6). Immunoblot analysis revealed that PYRIN-1.DELTA.PYRIN was expressed at levels similar to that of PYRIN-1, indicating that loss of function was not due to reduced protein levels (FIG. 15, upper panel). In contrast, deletion of the C-terminal domain showed a 2-fold increase in the synergistic activation of NF-kB relative to full-length protein suggesting that the LRRs may function as a negative regulator of PYRIN-1 activity FIG. 15, lane 8). To confirm that the synergistic effect was specific for PYRIN-1, ASC was co-expressed with CARD-9, a CARD-containing NF-kB activator (Bertin at al. (2000) J. Biol. Chem. 275:41082). When expressed alone, CARD-9 induced NF-kB activity 15-20-fold compared with empty vector (FIG. 15, lane 9). However, when CARD-9 and ASC were co-expressed, CARD-9 failed to synergistically activate ASC-induced NF-kB activity (FIG. 15, lane 10). Taken together, these data demonstrate that PYRIN-1 functions as an activator of ASC activity and is an upstream regulator of NF-kB signaling.

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DOCUMENT-IDENTIFIER: US 20020197616 A1

TITLE: Nod2 nucleic acids and proteins

PUBLICATION-DATE: December 26, 2002

INVENTOR-INFORMATION:

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RELATED-US-APPL-DATA:

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US-CL-CURRENT: 435/6

ABSTRACT:

The present invention relates to intracellular signalling molecules, in particular the Nod2 protein and nucleic acids encoding the Nod2 protein. The present invention provides isolated nucleotide sequence encoding Nod2, isolated Nod2 peptides, antibodies that specifically bind Nod2, methods for the detection of Nod2, and methods for screening compounds for the ability to alter Nod2 associated signal transduction. The present invention also provides Nod2 variant alleles. The present invention further provides methods of identifying individuals at increased risk of developing Crohn's disease.

[0001] This application claims priority to U.S. provisional patent applications serial Nos. 60/244,266 and 60/286,316, each of which is herein incorporated by reference in its entirety. This patent application was supported in part by grant CA-64556 from the National Institutes of Health. The government has certain rights in the invention.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (17):

[0046] FIG. 5 shows that Nod2 acts through the **IKK complex** to activate NF-.kappa.B.

Detail Description Paragraph - DETX (5):

[0104] Nod2 is the first molecule known to contain two CARDS. The molecular basis underlying the requirement of both CARDs of Nod2 for RICK binding remains unclear. The present invention is not limited to any particular mechanism of action. Indeed, an understanding of the mechanism of action is not necessary to practice the present invention. Nevertheless, it is contemplated that the presence of both CARDs may enhance the affinity for the CARD of RICK. Another possibility is that upon an initial interaction involving a CARD of Nod2 and the CARD of RICK, Nod2 may undergo a conformational change that allows the second CARD to associate with high affinity to RICK. The intermediate region of RICK associates with IKK.gamma. (Inohara et al., [2000], supra), providing a direct link between Nod1/Nod2 and the **IKK complex**. Consistent with this model, NF-.kappa.B activation induced by Nod2 as well as that induced by Nod I required IKK.gamma. and was inhibited by dominant negative forms of IKK.gamma., IKK.alpha. and IKK.beta.. The functional role for the LRRs of Nod1 and Nod2 remains unclear. The LRR is a repeated protein-protein interaction module that is presumably involved in the activation of Nod1 and Nod2 by upstream signals. In the case of plant NBD/LRR-containing R proteins, their LRRs appear to be important for the recognition of pathogen components and their N-terminal domains appear to mediate a signaling cascade that regulates gene expression (Parniske et al., supra, Dixon et al., supra). Because both Nod1 and Nod2 activate NF-.kappa.B, their LRRs may act to recognize a different set of intracellular stimuli that mediate Nod1 and Nod2 oligomerization and association with RICK. Because Nod2 is expressed primarily in monocytes, Nod2 might serve as an intracellular receptor that transduces signals in the monocyte/macrophage that lead to activation of and transcription of regulatory genes.

Detail Description Paragraph - DETX (344):

[0437] This example demonstrates that NF-.kappa.B activation induced by Nod2 requires IKK.gamma. and is inhibited by dominant negative forms of **IKKs** and RICK. A main pathway of NF-.kappa.B activation is mediated by I.kappa.B kinases (**IKKS**) resulting in I.kappa.B phosporylation and release of cytoplamic NF-.kappa.B (Karin, J. Biol. Chem. 274: 27339-27342 [1999]). To determine whether Nod2 activates an **IKK**-dependent pathway, Nod2 was co-expressed with mutant forms of IKK.alpha., IKK.gamma., and I.kappa.B.alpha. that have been shown to act as dominant inhibitors of their corresponding endogenous counterparts and/or the **IKK complex** (Karin, supra). In addition, a truncated mutant of IKK.gamma./Nemo (residues 134-419) was used that is defective in IKK.alpha. and IKK.beta. binding and acts as an inhibitor of NF-.kappa.B activation induced by RIP and RICK (Inohara et al., [2000], supra). The NF-.kappa.B activity induced by Nod2 as well as that induced by TNF.alpha. stimulation were greatly inhibited by mutant IKK.alpha., IKK.gamma.,

IKK.gamma., and I.kappa.B.alpha. (FIG. 5A). Because RICK has been shown to serve as a downstream target of Nod1 (Bertin et al., supra, Inohara et al., [1999] supra, Inohara et al., [2000], supra), a truncated form of RICK containing its CARD (residues 406-540) that acts as a dominant inhibitor of Nod1 activity (Bertin et al., supra) was used to test whether NF-.kappa.B activation induced by Nod2 is similarly inhibited by this RICK mutant. NF-.kappa.B activation induced by Nod2 was inhibited by mutant RICK but not by a mutant form of RIP that expresses its death effector domain (FIG. 5A). The inhibition by the CARD of RICK was specific in that it did not interfere with ability of TNF.alpha. to induce NF-.kappa.B, an activity that was inhibited by the RIP mutant (FIG. 5A). To verify that Nod2 acts upstream of the **IKK complex** to activate NF-.kappa.B, we tested the ability of Nod2 to activate NF-.kappa.B in parental Ratl fibroblasts and 5R cells, a Ratl derivative cell line that is defective in IKK.gamma., an essential subunit of the **IKKs** (Yamaoka et al., supra). Nod2, as well as Nod1 and TNF.alpha. oc, induced NF-.kappa.B activity in parental Ratl cells but not in IKK.gamma.-deficient 5R cells (FIG. 5B). As a control, expression of IKK.beta., which functions downstream of IKK.gamma., induced NF-.kappa.B activation in both Ratl and 5R cell lines (FIG. 5B). These results indicate that Nod2 acts through IKK.gamma./**IKK**/IKK.beta. to activate NF-.kappa.B.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020193284 A1

TITLE: Methods for identifying modulators of NF-KB activity

PUBLICATION-DATE: December 19, 2002

INVENTOR-INFORMATION:

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APPL-NO: 09/ 884875

DATE FILED: June 18, 2001

US-CL-CURRENT: 514/1, 435/18

ABSTRACT:

The present invention provides methods for identification of agents that modulate NF-.kappa.B activity through modulation of the acetylation and deacetylation of the RelA subunit of NF-.kappa.B.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (6):

[0004] The prototypical NF-.kappa.B **complex,** which corresponds to a heterodimer of p50 and RelA subunits, is kept in an inactive form and sequestered in the cytoplasm by a family of inhibitory proteins termed the I.kappa.Bs, which includes I.kappa.B.alpha.. Upon exposure to a wide variety of stimuli, for example proinflammatory cytokines like tumor necrosis factor a (TNF-.alpha.), and interleukin 1 (IL-1), I.kappa.B.alpha. is phosphorylated. This event, mediated by a macromolecular I.kappa.B kinase **complex (IKK)** (Karin, M., Oncogene 18:6867-6874 (1999)), triggers the rapid ubiquitination and subsequent degradation of this inhibitor by the 26S proteasome **complex**. The unmasking of the nuclear localization signal in the RelA component of the p50/RelA NF-.kappa.B heterodimer allows its rapid translocation into the nucleus, where it engages cognate .kappa.B sites and activates transcription of various target genes.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020187922 A1

TITLE: Novel molecules of the pyrin domain protein family and uses thereof

PUBLICATION-DATE: December 12, 2002

INVENTOR-INFORMATION:

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APPL-NO: 10/ 127516

DATE FILED: April 22, 2002

RELATED-US-APPL-DATA:

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parent continuation-in-part-of 09653901 20000901 US PENDING

child 09653901 20000901 US

parent continuation-in-part-of 09506067 20000217 US ABANDONED

US-CL-CURRENT: 514/1, 435/7.23

ABSTRACT:

Diagnostic, screening and therapeutic methods utilizing NBS-1 and PYRIN-1 are disclosed. The methods take advantage of the interactions between NBS-1 or PYRIN-1 and various proteins involved in apoptotic and inflammatory signaling pathways. Also disclosed are methods for identifying modulators of ASC and NF-.kappa.B.

RELATED APPLICATIONS

[0001] This application is a continuation-in-part and claims the benefit of

priority under 35 U.S.C. 120 of U.S. application Ser. No. 10/027,629, filed on Dec. 20, 2001, which is a continuation-in-part and claims the benefit of priority of U.S. application Ser. No. 09/964,955, filed on Sep. 26, 2001, which is a continuation-in-part and claims the benefit of priority of U.S. application Ser. No. 09/653,901, filed on Sep. 1, 2000; which is a continuation-in-part of U.S. application Ser. No. 09/506,067, filed on Feb. 17, 2000. The disclosure of the prior applications are considered part of and incorporated by reference in the disclosure of this application.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (17):

[0057] FIG. 16 depicts the results of an NF-kB activity assay showing that PYRIN-1 and ASC activate NF-kB through the **IKK complex**. In this study 293T cells were transfected with a plasmid expressing ASC (32 ng) and a plasmid expressing PYRIN-1 (500 ng) and either empty vector (500 ng) or a plasmid expressing dominant negative mutants of IKK.gamma. (IKK.gamma.-DN), or **IKK2 (IKK2**-DN). Immunoblot analysis was performed to monitor expression of PYRIN-1 (upper panel) and ASC (middle panel). Relative luciferase activities were measured as described (lower panel).

Detail Description Paragraph - DETX (43):

[0096] NF-kB signaling occurred through the **IKK complex** because dominant-negative versions of **IKK**-.gamma. and **IKK-2** blocked the ability of PYRIN-1 to synergistically activate NF-kB FIG. 26, lanes 3 and 4). To determine the role of individual domains in NF-kB signaling, the ability of the PYRIN-1 truncation mutants to activate NF-kB was investigated. The N-terminal PYRIN domain of PYRIN-1 was essential for NF-kB signaling, since deletion of this domain (PYRIN-1.DELTA.PYRIN) eliminated the synergistic induction of NF-kB activity (FIG. 15, lane 6). Immunoblot analysis revealed that PYRIN-1.DELTA.PYRIN was expressed at levels similar to that of PYRIN-1, indicating that loss of function was not due to reduced protein levels (FIG. 15, upper panel). In contrast, deletion of the C-terminal domain showed a 2-fold increase in the synergistic activation of NF-kB relative to full-length protein suggesting that the LRRs may function as a negative regulator of PYRIN-1 activity (FIG. 15, lane 8). To confirm that the synergistic effect was specific for PYRIN-1, ASC was co-expressed with CARD-9, a CARD-containing NF-kB activator (Bertin at al. (2000) J. Biol. Chem. 275:41082). When expressed alone, CARD-9 induced NF-kB activity 15-20-fold compared with empty vector (FIG. 15, lane 9). However, when CARD-9 and ASC were co-expressed, CARD-9 failed to synergistically activate ASC-induced NF-kB activity (FIG. 15, lane 10). Taken together, these data demonstrate that PYRIN-1 functions as an activator of ASC activity and is an upstream regulator of NF-kB signaling.

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DOCUMENT-IDENTIFIER: US 20020183399 A1

TITLE: Method and compositions for treating rosacea

PUBLICATION-DATE: December 5, 2002

INVENTOR-INFORMATION:

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Fisher, Gary J.	Ypsilanti	MI	US	

APPL-NO: 10/ 142724

DATE FILED: May 9, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60289758 20010509 US

US-CL-CURRENT: 514/725, 514/559 , 514/703

ABSTRACT:

Rosacea is treated with a composition comprising an antimicrobial and at least one of an anti-inflammatory and a non-retinoid inhibitor of at least one of NF-k.beta., AP-1, MMPs, adhesion molecules, TLRs, and CD14. The composition may further comprise a retinoid.

---------- KWIC ---------

Detail Description Paragraph - DETX (17):

[0031] NF-.kappa.B inhibitors include those disclosed in the following references. Cyclopentenone prostaglandins: Rossi et al., "Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase", Nature, vol. 403, no. 6765, Jan. 6, 2000 (pp. 103-8). Quercetin and staurosporine: Peet and Li, "IkappaB kinases alpha and beta show a random sequential kinetic mechanism and are inhibited by staurosporine and quercetin", J. Biol Chem, vol. 274, no. 46, Nov. 12, 1999 (pp. 32655-61) (but not the quercetin analogue Daidzein). Nepalolide A: Wang et al., "Nepalolide A inhibits the expression of inducible nitric oxide synthase by modulating the degradation of IkappaB-alpha and IkappaB-beta in C6 glioma cells and rat primary astrocytes", Br J. Pharmacol, vol. 128, no. 2, September 1999 (pp. 345-56). Turmeric (curcumin): Plummer et al., "Inhibition of cyclo-oxygenase 2 expression in colon cells by

the chemopreventive agent curcumin involves inhibition of NF-kappaB activation via the NIK/**IKK** signalling **complex**", Oncogene, vol. 18, no. 44, Oct. 28, 1999 (pp. 6013-20). Salicylates: Stevenson et al., "Salicylic acid and aspirin inhibit the activity of RSK2 kinase and repress RSK2-dependent transcription of cyclic AMP response element binding protein- and NF-kappa B-responsive genes", J. Immunol, vol. 163, no.10, Nov. 15, 1999 (pp. 5608-16). Diterpenes: de las Heras et al., "Inhibition of NOS-2 expression in macrophages through the inactivation of NF-kappaB by andalusol", Br J. Pharmacol, vol. 128, no. 3, October 1999 (pp. 605-12) (andalusol, ent-6.alpha.,8.alpha.,18-trihydroxy-13(16),- 14-labdadiene, is a naturally occurring diterpene, isolated from Sideritis foetens (Lamiaceae). N-substituted benzamides: Liberg et al., "N-substituted benzamides inhibit NFkappaB activation and induce apoptosis by separate mechanisms", Br J. Cancer, vol. 81, no.6, November 1999 (pp. 981-8). While not preferred due to potential toxicity issues, arsenic: Estrov et al., "Phenylarsine oxide blocks interleukin-1.beta.-induced activation of the nuclear transcription factor NF-KB, inhibits proliferation, and induces apoptosis of acute myelogenous leukemia cells", Blood, vol. 94, no. 8, Oct. 15, 1999 (pp. 2844-53). Genistein: Tabary et al., "Genistein inhibits constitutive and inducible NFkappaB activation and decreases IL-8 production by human cystic fibrosis bronchial gland cells", Am J. Pathol, vol. 155, no. 2, August 1999 (pp. 473-81). Theophylline: Tomita et al., "Functional assay of NF-kappaB translocation into nuclei by laser scanning cytometry: inhibitory effect by dexamethasone or theophylline", Naunyn Schmiedebergs Arch Pharmacol, vol. 359, no. 4, April 1999 (pp. 249-55). Cepharanthine: a plant alkaloid (I) (Merck Index 11, 306,1981), and described in U.S. Pat. Nos. 2,206,407 and 2,248,241, and Japanese Patents 120,483,128,533, and 141,292. Trifluoroalkyl salicylates: Bayon et al., "4-trifluoromethyl derivatives of salicylate, triflusal and its main metabolite 2-hydroxy-4- trifluoromethylbenzoic acid, are potent inhibitors of nuclear factor kappaB activation", Br J. Pharmacol, vol. 126, no. 6, March 1999 (pp. 1359-66) (2- hydroxy-4-trifluoromethylbenzoic acid (HTB) and 2-acetoxy-4-trifluoromethylbenzoic acid (triflusal), both more potent than aspirin or salicylate as inhibitors of NF-.kappa.B, indicating that the incorporation of a 4-trifluoromethyl group to the salicylate molecule strongly enhances its inhibitory effect on NF-.kappa.B activation). Quinapril: quinapril hydrochloride is chemically described as [3S-[2[R*(R*)],3R*]]-2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-ox-opropyl]-1,2,3,4-tetrahydro-3- isoquinolinecarboxylic acid, monohydrochloride. Its empirical formula is C.sub.25H.sub.30N.sub.2O.sub.- 5 .multidot.HCl . Cyclosporine A: Meyer et al., "Cyclosporine A is an uncompetitive inhibitor of proteasome activity and prevents NF-kappaB activation", FEBS Lett, vol. 413, no. 2, Aug. 18, 1997 (pp. 354-8). Arachidonic acid derivatives: Thommensen et al., "Selective inhibitors of cytosolic or secretory phospholipase A2 block TNF-induced activation of transcription factor nuclear factor-kappa B and expression of ICAM-1 ", J. Immunol, vol. 161, no. 7, Oct. 1, 1998 (pp. 3421-30) (TNF-induced activation of NF-.kappa.B inhibited by trifluoromethyl ketone analogue of arachidonic acid (AACOCF.sub.3), methyl arachidonyl fluorophosphate, trifluoromethyl ketone analogue of eicosapentaenoic acid (EPACOCF.sub.3), 12-epi-scalaradial, and LY311727; arachidonyl methyl ketone analogue (AACOCH.sub.3) and the eicosapentanoyl analogue (EPACHOHCF.sub.3) had no effect on TNF-induced NF-.kappa.B activation. Genistein, erbstatin: Natarajan et aL, "Protein tyrosine kinase inhibitors block tumor necrosis factor-induced activation of nuclear factor-KB, degradation of IKB.alpha.,

nuclear translocation of p65, and subsequent gene expression", Arch Biochem Biophys, vol. 352, no. 1, Apr. 1, 1998 (pp. 59-70). Fasudil: 1-(5-isoquinolinesulfonyl)homopiperazine hydrochloride (fasudil hydrochloride); Sato et al., "Inhibition of human immunodeficiency virus type 1 replication by a bioavailable serine/threonine kinase inhibitor, fasudil hydrochloride", AIDS Res Hum Retroviruses, vol. 14, no. 4, Mar. 1, 1998 (pp. 293-8). ACE (angiotensin converting enzyme) inhibitors, like quinipril: Hernandez- Presa et al., "Angiotensin-converting enzyme inhibition prevents arterial nuclear factor-kappa B activation, monocyte chemoattractant protein-1 expression, and macrophage infiltration in a rabbit model of early accelerated atherosclerosis'8 , Circulation, vol. 95, no. 6, Mar. 18, 1998 (pp. 1532-41). Synthetic 1,3,7-trialkyl xanthine derivatives, such as pentoxifylline (3,7-dimethyl-1-(5-oxohexyl)xanthine; Drugs & Aging 1995, 7/6: 480-503) and denbufylline (1,3-dibutyl-7-(2-oxopropyl)xanthine); Lee et al., "Pentoxifylline blocks hepatic stellate cell activation independently of phosphodiesterase inhibitory activity", Am J. Physiol, vol. 273, no. 5 Pt 1, November 1997 (pp. G1094-100). Benzophenanthradine derivatives: Chaturvedi et al, "Sanguinarine (pseudochelerythrine) is a potent inhibitor of NF-.kappa.B activation, I.kappa.B.alpha. phosphorylation, and degradation", J. Biol Chem, vol. 272, no. 48, Nov. 28, 1997 (pp. 30129-34) (sanguinarine, a benzophenanthridine alkaloid). Actinomycin D: Faggioli et al., "Protein synthesis inhibitors cycloheximide and anisomycin induce interleukin-6 gene expression and activate transcription factor NF-.kappa.B", Biochem Biophys Res Commun, vol. 233, no. 2, Apr. 17, 1997 (pp. 507-13) (IL-6 mRNA accumulation in two human cell lines, MDA-MB-231 and HeLa, stimulated by cycloheximide or anisomycin is almost completely inhibited in the presence of actinomycin D). Hydroxyanthranilic acids: Sekkai et al., "Inhibition of nitric oxide synthase expression and activity in macrophages by 3-hydroxyanthranilic acid, a tryptophan metabolite", Arch Biochem Biophys, vol. 340, no. 1, Apr. 1, 1997 (pp. 117-23) (3-hydroxyanthranilic acid but not anthranilic acid). Nordihydroguaiaretic acid and AA861: Lee et al., "Inhibition of 5- lipoxygenase blocks IL-1 beta-induced vascular adhesion molecule-1 gene expression in human endothelial cells", J. Immunol, vol. 158, no. 7, Apr 1, 1997 (pp. 3401-7). Prostaglandin Al: Rossi et al., "Inhibition of nuclear factor kappa B by prostaglandin A1: an effect associated with heat shock transcription factor activation", Proc Natl. Acad Sci USA, vol. 94, no. 2, Jan. 21, 1997 (pp. 746-50).

PGPUB-DOCUMENT-NUMBER: 20020169299

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DOCUMENT-IDENTIFIER: US 20020169299 A1

TITLE: Gene for identifying individuals with familial dysautonomia

PUBLICATION-DATE: November 14, 2002

INVENTOR-INFORMATION:

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APPL-NO: 10/ 041856

DATE FILED: January 7, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60260080 20010106 US

US-CL-CURRENT: 536/23.1

ABSTRACT:

This invention relates to methods and compositions useful for detecting mutations which cause Familial Dysautonomia. Familial dysautonomia (FD; Riley-Day syndrome), an Ashkenazi Jewish disorder, is the best known and most frequent of a group of congenital sensory neuropathies and is characterized by widespread sensory and variable autonomic dysfunction. Previously, we mapped the FD gene, DYS, to a 0.5 cM region of chromosome 9q31 and showed that the ethnic bias is due to a founder effect, with >99.5% of disease alleles sharing a common ancestral haplotype. To investigate the molecular basis of FD, we sequenced the minimal candidate region and cloned and characterized its 5 genes. One of these, IKBKAP, harbors two mutations that can cause FD. The major haplotype mutation is located in the donor splice site of intron 20. This mutation can result in skipping of exon 20 in the mRNA from FD patients, although they continue to express varying levels of wild-type message in a tissue-specific manner. RNA isolated from patient lymphoblasts is primarily wild-type, whereas only the deleted message is seen in RNA isolated from brain. The mutation associated with the minor haplotype in four patients is a missense (R696P) mutation in exon 19 that is predicted to disrupt a potential phosphorylation site. Our findings indicate that almost all cases of FD are caused by an unusual splice defect that displays tissue-specific expression; and they also provide the basis for rapid carrier screening in the Ashkenazi Jewish population.

[0001] This application claims priority to provisional application Serial No.

60/260,080, the entirety of which is incorporated herein by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (3):

[0046] These findings have direct implications for understanding the clinical manifestations of FD, for preventing it and potentially for treating it. The IKAP protein produced from IKBKAP gene was originally isolated as part of a large interleukin-1-inducible **IKK complex** and described as a regulator of kinases involved in pro-inflammatory cytokine signaling (Cohen et al. 1998). However, a recent report questioned this conclusion, by reporting that cellular **IKK** complexes do not contain IKAP based on various protein-protein interaction and functional assays. Rather, IKAP appears to be a member of a novel **complex** containing additional unidentified proteins of 100, 70, 45, and 39 kDa (Krappmann et al. 2000).

Detail Description Paragraph - DETX (86):

[0127] Comparison of the FD and control sequences revealed 152 differences (excluding simple sequence repeat markers), which include 26 variations in the length of dT.sub.n tracts, 1 VNTR, and 125 base pair changes. Each of the 125 base pair changes was tested in a panel of 50 individuals known to carry two non-FD chromosomes by segregation in FD families. Of the 125 changes tested, only 1 was unique to patients carrying the major FD haplotype. This T-C change is located at bp 6 of intron 20 in the IKBKAP gene depicted in FIG. 1, and is demonstrated in FIG. 2A. IKAP was originally identified as an I.kappa.B kinase (**IKK) complex**-associated protein that can bind both NF-.kappa.B inducing kinase (MK) and **IKKs** through separate domains and assemble them into an active kinase **complex** (Cohen et al. 1998). Recent work, however, has shown that IKAP is not associated with **IKKs** and plays no specific role in cytokine-induced NF-.kappa.B signaling (Krappmann et al. 2000). Rather, IKAP was shown to be part of a novel multi-protein **complex** hypothesized to play a role in general transcriptional regulation.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020168683 A1

TITLE: Human pellino polypeptides

PUBLICATION-DATE: November 14, 2002

INVENTOR-INFORMATION:

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APPL-NO: 09/ 843905

DATE FILED: April 27, 2001

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non-provisional-of-provisional 60200198 20000428 US

US-CL-CURRENT: 435/7.1, 435/320.1 , 435/325 , 435/69.1 , 530/350 , 536/23.5

ABSTRACT:

There are disclosed novel polypeptides referred to as Pellino polypeptides, as well as fragments thereof, including immunogenic peptides. DNAs encoding such polypeptides as well as methods of using such DNAs and polypeptides are also disclosed.

[0001] This application claims the benefit under 35 U.S.C. 119(e) of U.S. provisional application Serial No. 60/200,198, filed Apr. 28, 2000, which is incorporated by reference herein.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (6):

[0005] Downstream components of the Toll signaling pathway have also been evolutionarily conserved in mammalian TLR and interleukin-1 receptor signaling pathways which culminate in nuclear translocation of the transcription factor Nuclear Factor kappa B (NF-kB). Protein kinases IRAK-1 and IRAK2, close homologues of Pelle, are recruited to the activated IL-1 R or TLR receptor complexes through the adaptor protein MyD88 and undergo autophosphorylation reactions. Although MyD88 is not a strict analog of Tube, both proteins contain a so-called death domain, and Tube likely serves to mediate signal transmission between Toll and Pelle, to which it binds. IRAK subsequently

interacts with another adaptor molecule TRAF-6, which is homologous to the recently described D-TRAF. Signals downstream of TRAF appear to be divergent, and not all of them are fully understood, but one consequence, in mammalian cells, is the activation of the IkB kinase (**IKK) complex** which directly phosphorylates the inhibitory Cactus homolog IkB at two N-terminal serine residues causing its ubiquitination and degradation. Released from a cytoplasmic association with IkB, NF-kB migrates into the nucleus. Recently, a candidate for an additional intermediate in Tube-Pelle interactions was found by yeast two-hybrid screening with Pelle as a bait sequence. This protein, called Pellino, was shown to interact with catalytically-competent Pelle, but not with a mutant form of Pelle that lacked kinase activity. Although a function for Pellino was not addressed in this study, it was suggested that it could either stabilize the activated form of Pelle, or mediate an interaction with downstream Pelle substrates.

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DOCUMENT-IDENTIFIER: US 20020168656 A1

TITLE: Detection of mutations in a gene encoding IkappaB kinase-complex-associate- d protein to diagnose familial dysautonomia

PUBLICATION-DATE: November 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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APPL-NO: 10/ 050189

DATE FILED: January 16, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60262284 20010117 US

US-CL-CURRENT: 435/6

ABSTRACT:

A method for detecting the presence in a subject of a polymorphism linked to a gene associated with familial dysautonomia, said method comprising detecting a disruptive mutation in a gene of said subject encoding the I.kappa.B kinase-complex-associated protein, and, preferably, detecting a T.fwdarw.C change in position 6 of the donor splice site of intron 20 and/or a G.fwdarw.C transversion of nucleotide 2390 in exon 19 of the gene encoding the I.kappa.B kinase-complex-associated protein which is present on chromosome 9q31. Also disclosed are oligonucleotide primers useful in the detection method. This abstract is provided to comply with the rules requiring an abstract that will allow a searcher or other reader to ascertain quickly the subject matter of the technical disclosure. It is submitted with the understanding that it will not be used to interpret or limit the scope or meaning of the claims. 37 CFR .sctn.1.72(b).

---------- KWIC ---------

Detail Description Paragraph - DETX (10):

[0025] IKAP was initially identified and named based on its reported ability to bind the I.kappa.B kinases (**IKKs**), the NF-.kappa.B inhibitory subunit

I.kappa.B-.alpha., NF-.kappa.B and the NF-.kappa.B-inducing kinase (NIK) and assemble these proteins into an active kinase **complex** (9). Recent studies, however, suggest that IKAP is not associated with the **IKKs** and plays no specific role in cytokine-induced NF-.kappa.B activation (10).
Characterization of the amino acid sequence of IKAP reveals significant amino acid sequence homology with the Saccharomyces cerevisiae IKI3 (11) and ELP1 (12) proteins as well as similar proteins in Schizosaccharomyces pombe and Arabidopsis thaliana. The IKI3 gene product mediates, by a yet to be determined mechanism, sensitivity to the yeast killer toxin (11). ELP1 is a subunit of a multisubunit **complex** that is associated with RNA polymerase II and is required for the activation and transcriptional elongation of a large number of genes (12). If IKAP, like ELP 1, is a part of the RNA polymerase II elongation **complex** and plays a role in gene activation, the absence of functional IKAP in FD-affected individuals may prevent gene activation events necessary for normal neuronal development and function.

PGPUB-DOCUMENT-NUMBER: 20020162126

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020162126 A1

TITLE: Methods and compositions for RNA interference

PUBLICATION-DATE: October 31, 2002

INVENTOR-INFORMATION:

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Hannon, Gregory	Huntington	NY	US	

APPL-NO: 09/ 866557

DATE FILED: May 24, 2001

RELATED-US-APPL-DATA:

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parent continuation-in-part-of PCT/US01/08435 20010316 US UNKNOWN

non-provisional-of-provisional 60189739 20000316 US

non-provisional-of-provisional 60243097 20001024 US

US-CL-CURRENT: 800/8, 435/455 , 514/44

ABSTRACT:

The present invention provides methods for attenuating gene expression in a cell using gene-targeted double stranded RNA (dsRNA). The dsRNA contains a nucleotide sequence that hybridizes under physiologic conditions of the cell to the nucleotide sequence of at least a portion of the gene to be inhibited (the "target" gene).

RELATED APPLICATIONS

[0001] This application is a continuation-in-part of PCT application PCT/US01/08435, filed Mar. 16, 2001, and claims the benefit of U.S. Provisional applications U.S. Ser. No. 60/189,739 filed Mar. 16, 2000 and U.S. Ser. No. 60/243,097 filed Oct. 24, 2000. The specifications of such applications are incorporated by reference herein.

---------- KWIC ---------

Detail Description Paragraph - DETX (10):

[0078] In certain embodiments, the cells can be treated with an agent(s) that inhibits the general double-stranded RNA response(s) by the host cells, such as may give rise to sequence-independent apoptosis. For instance, the cells can be treated with agents that inhibit the dsRNA-dependent protein kinase known as PKR (protein kinase RNA-activated). Double stranded RNAs in mammalian cells typically activate protein kinase PKR and leads to apoptosis. The mechanism of action of PKR includes phosphorylation and inactivation eIF2a (Fire (1999) Trends Genet 15:358). It has also been reported that induction of NF-.kappa.B by PKR is involved in apoptosis commitment and this process is mediated through activation of the **IKK complex**. This sequence-independent response may reflect a form of primitive immune response, since the presence of dsRNA is a common feature of many viral lifecycles.

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TITLE: Antiinflammation agents

PUBLICATION-DATE: October 31, 2002

INVENTOR-INFORMATION:

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DATE FILED: October 23, 2001

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US-CL-CURRENT: 514/228.2, 514/233.8 , 514/252.13 , 514/252.16 , 514/258.1 , 514/266.2 , 514/307 , 514/314 , 544/116 , 544/284 , 544/60 , 546/144 , 546/167

ABSTRACT:

Compounds, compositions and methods that are useful in the treatment of inflammatory, immunoregulatory, metabolic and cell proliferative conditions or diseases are provided herein. In particular, the invention provides compounds which modulate the expression and/or function of proteins involved in inflammation, metabolism and cell proliferation. The subject compounds contain fused carbocyclic or heterocyclic rings.

[0001] This application claims the benefit of U.S. Ser. No. 60/243,582 filed Oct. 26, 2000 which is incorporated by reference in its entirety

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (5):

[0005] In its inactive state, the NF-.kappa.B heterodimer is held in the cytoplasm by association with inhibitory IkB proteins. Recently, the three-dimensional structure of a NF-.kappa.B/I.kappa.B ternary **complex** has been solved (Huxford et al. Cell, 95, 759 (1998); Jacobs et al. Cell, 95, 749 (1998)). When cells are treated with the appropriate stimuli, such as IL-1 or TNF, intracellular signal transduction pathways are activated that lead to the eventual phosphorylation of IkB proteins on two specific residues (serines 32 and 36 in IkB.alpha., serines 19 and 23 in IkB). Mutation of one or both serine residues renders IkB resistant to cytokine-induced phosphorylation. This signal-induced phosphorylation targets IkB for ubiquitination and proteosome-mediated degradation, allowing nuclear translocation of NF-.kappa.B (Thanos and Maniatis, Cell, 80, 529 (1995)). The only regulated step in the IkB degradation pathway is the phosphorylation of IkB by IkB kinases (**IKK**) (Yaron et al. EMBO J. 16, 6486 (1997)).

Summary of Invention Paragraph - BSTX (6):

[0006] Several intermediate steps in the TNF- and IL-1-activated signaling pathways that result in IkB phosphorylation have been elucidated in recent years. Both pathways appear to merge at the level of the protein kinase NIK (NF-.kappa.B-inducing kinase) (Malinin et al. Nature, 385, 540 (1997); Song et al Proc. Natl Acad. Sci. USA, 94, 9792 (1997)). Similarly, the protein kinases MEKK1 and MLK3 have been implicated in the induction of **IKK** activity (Lee et al. Proc. Natl. Acad. Sci. USA. 95, 9319 (1998); Hehner et al. Mol. Cell. Biol. 20, 2556 (2000)). While the specific details remain somewhat unclear regarding how these or other intermediate proteins may interact with and/or stimulate **IKK** activity in cells, significant progress has been made in elucidating the enzymes responsible for IkB phosphorylation. Two **IKK** enzymes, generally referred to as IKK.alpha. and IKK.beta. (Woronicz et al. Science, 278, 866 (1997); Zandt et al. Cell, 91, 243 (1997)) or **IKK-1 and IKK-2** (Mercurio et al. Science, 278, 860 (1997)) have been discovered. Both forms of **IKK** can exist as homodimers and as IKK.alpha./IKK.beta. heterodimers. Another recently discovered component of the IkB kinase **complex** is a regulatory protein, known as **IKK**-gamma or NEMO (NF-.kappa.B-Essential Modulator) (Rothwarf et al. Nature, 395, 297 (1998)). NEMO does not contain a catalytic domain, and thus it appears to have no direct kinase activity and it probably serves a regulatory function. Existing data suggest that the predominant form of **IKK** in cells is an IKK.alpha./IKK.beta. heterodimer associated with either a dimer or a trimer of NEMO (Rothwarf et al. Nature 395, 297 (1998)).

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TITLE: Protein variants

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INVENTOR-INFORMATION:

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DATE FILED: December 19, 2001

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non-provisional-of-provisional 60115010 19990106 US

US-CL-CURRENT: 514/12

ABSTRACT:

IGF-I variants having an alanine, glycine, or serine amino acid residue at position 16, 25, 49 or at positions 3 and 49 of native-sequence IGF-I are provided that are useful to treat a disorder characterized by dysregulation of the GH/IGF axis in a mammal, such as a renal disorder.

RELATED APPLICATIONS

[0001] This application is a divisional of application Ser. No. 09/477,924 filed Jan. 5,2000, which is a non-provisional application filed under 37 CFR 1.53(b)(1), claiming priority under 35 USC .sctn.119 to provisional application no. 60/170,261 filed Dec. 9, 1999 and provisional application No. 60/115,010 filed Jan. 6, 1999, the entire disclosures of which are hereby incorporated by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (25):

[0025] The dynamics of IGF-I interaction with sensitive tissues are **complex** and incompletely understood. Biological activity of circulating IGF-I is regulated by levels of plasma IGFBPs, which both enhance and inhibit IGF-I actions (Cohick and Clemmons, Annu. Rev. Phvsiol. 55: 131-153 (1993); Kupfer et al., J. Clin. Invest. 91: 391-396 (1993)). In addition, IGFBPs present in tissues regulate the interaction of circulating IGF-I with its receptor. Tissue IGF-I receptor density is altered by changes in levels of circulating IGF-1. In kidney, the numbers of IGF-I receptors are inversely related to levels of circulating IGF-I (Hise et al., Clin. Sci., 83: 233-239 (1992)). It is known that under some circumstances elevated levels of circulating IGF-I are associated with or directly causative of long-term changes in renal function. For example, the enhancements of insulin and PAH clearances that accompany the elevations of circulating GH and IGF-I in patients with acromegaly are sustained over years of time (**Ikkos** et al., Acta Endocrinol. 21: 226-236 (1956)). An increase in creatinine clearance occurred within the first 12 days of IGF-I administration to a GH-insensitive Laron dwarf. The increase was progressive over the next 59 days (Walker et al., J. Pediatr. 121: 641-646 (1992)).

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020156000 A1

TITLE: Anti-inflammatory compounds and uses thereof

PUBLICATION-DATE: October 24, 2002

INVENTOR-INFORMATION:

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DATE FILED: May 2, 2001

RELATED-US-APPL-DATA:

child 09847940 A1 20010502

parent continuation-in-part-of 09643260 20000822 US PENDING

non-provisional-of-provisional 60201261 20000502 US

US-CL-CURRENT: 514/12

ABSTRACT:

The present invention provides anti-inflammatory compounds, pharmaceutical compositions thereof, and methods of use thereof for treating inflammatory disorders. The present invention also provides methods of identifying anti-inflammatory compounds and methods of inhibiting NF-.kappa.B-dependent target gene expression in a cell.

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 60/201,261 filed May 2, 2000 and to U.S. patent application Ser. No. 09/643,260 filed Aug. 22, 2000, the entire contents of each of which are incorporated herein by reference.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (6):

[0028] FIG. 5 depicts results from experiments indicating that a

cell-permeable peptide spanning the IKK.beta. NBD inhibits the IKK.beta./NEMO interaction, TNF.alpha.-induced NF-.kappa.B activation and NF-.kappa.B-dependent gene expression. (A) GST-pull-down analysis was performed using either GST-NEMO-in vitro translated IKK.beta. (upper panel) or GST-IKK.beta.-(644-756)-in vitro translated NEMO (lower panel). The assay was performed in the absence (no peptide) or presence of increasing concentrations (125, 250, 500 or 1000 .mu.M) of either mutant (MUT) or wild-type (WT) NBD peptide. (B) HeLa cells were incubated with either peptide (200 .mu.M) for the times indicated. Following lysis, the **IKK complex** was immunoprecipitated using anti-NEMO and the resulting immunoblot probed with anti-IKK.beta.. (C) HeLa cells were transfected for forty-eight hours with pBIIX-luciferase then incubated for two hours in the absence (control) or presence of mutant or wild-type NBD peptide (100 and 200 .mu.M of each). Subsequently the cells were either treated with TNF.alpha. (10 ng/ml) as indicated (left panel) or left untreated (right panel) for a further four hours after which NF-.kappa.B activation was measured by luciferase assay. (D) HeLa cells were incubated for three hours with increasing concentrations (50, 100 or 200 .mu.M) of each peptide followed by treatment for fifteen minutes with TNF.alpha. (10 ng/ml) as indicated (+). Following lysis, nuclear extracts were made and 10 .mu.g of protein from each sample was used for EMSA using a specific [.sup.32P]-labeled .kappa.B-site probe. (E) Primary HUVEC were pre-incubated for two hours with of wild-type (left) or mutant (right) NBD peptides (100 .mu.M) then stimulated with TNF.alpha. (10 ng/ml) for a further six hours. Control cells received no peptide. Cells were stained with either anti-E-selectin (H4/18) or a non-binding control antibody (K16/16) and expression was measured by FACS (FACSort, Becton Dickinson). The profiles show E-selectin staining in the absence (shaded) and presence (solid line) of TNF.alpha. and control antibody staining under the same conditions (dashed line, no TNF.alpha.; dotted line, TNF.alpha.).

Detail Description Paragraph - DETX (14):

[0048] As used herein, the term "I.kappa.B-kinase" or "I.kappa.B protein kinase" or "I.kappa.B-kinase **complex**" or "I.kappa.B protein kinase **complex" or "IKK**" refers to a kinase that phosphorylates I.kappa.Bs.

Detail Description Paragraph - DETX (133):

[0164] These results establish that the interaction domain lies between residues 44 and 86, a region including the first a-helix of NEMO. A mutant was therefore made in which .alpha.-helix up to the first coiled-coil domain was deleted (residues T50-L93; del..alpha.H). This mutant did not interact with IKK.beta.-(644-756) (FIG. 2B). Furthermore transfection studies using pBIIX-luciferase demonstrated that del.aH inhibited TNF.alpha.-induced NF-.kappa.B activity (FIG. 2C) confirming previous reports that the COOH-terminus of NEMO which can not interact with IKK.beta., is a dominant-negative inhibitor of NF-.kappa.B (Mercurio et al., (1999) Mol. Cell. Biol. 19, 1526-1538; Rothwarf et al., (1998) Nature 395, 297-300). Taken together, FIGS. 1 and 2 show that the interaction between IKK.beta. and NEMO occurs via the COOH-terminus of IKK.beta. and the first .alpha.-helical region of NEMO. These findings suggest a model in which the NH2-terminus of NEMO

anchors it to the **IKK-complex** leaving the remainder of the molecule containing several protein:protein interaction domains free and accessible for interacting with upstream regulators of **IKK** function.

Detail Description Paragraph - DETX (140):

[0169] These results demonstrate that basal auto-phosphorylation and kinase activity of IKK.beta. is dependent on the ability of NEMO to associate with the kinase. One explanation for these observations may be that NEMO recruits a phosphatase to the **IKK-complex** that regulates basal IKK.beta. function by targeting the serine-rich region of the COOH-terminus. Inability to bind NEMO therefore prevents phosphatase recruitment and causes increased phosphorylation within this region.

Detail Description Paragraph - DETX (142):

[0171] An additional band representing a phosphorylated protein appeared only in the samples from TNF.alpha.-induced IKK.beta. (WT) and IKK.beta.-(1-744) transfected cells (FIG. 3F). The molecular weight of this protein (49 kDa) strongly suggests that it is endogenous NEMO associated with the precipitated **complex**. This is supported by the absence of the band in either precipitate (+/- TNF.alpha.) from IKK.beta.-(1-733) transfected cells. This protein has been identified as phosphorylated NEMO by dissociating the precipitated **complex** in SDS and re-immunoprecipitating [.sup.32P]-labeled NEMO using specific anti-NEMO antibodies. Induced phosphorylation of NEMO may therefore represent a further level of regulation of the activity of the **IKK complex**.

Detail Description Paragraph - DETX (155):

[0178] The relatively small size of the NBD makes it an attractive target for the development of compounds aimed at disrupting the core **IKK complex**. The relevance of this approach was investigated by designing cell-permeable peptides spanning the IKK.beta. NBD and determining their ability to dissociate the IKK.beta.-NEMO interaction.

Detail Description Paragraph - DETX (157):

[0180] The wild-type NBD peptide consisted of the region from T735 to E745 of IKK.beta. fused with a sequence derived from the third helix of the antennapedia homeodomain that has been shown to mediate membrane translocation (Derossi et al., (1994) J. Biol. Chem. 269, 10444-10450). The mutant was identical except that the tryptophan residues (W739 and W741) in the NBD were mutated to alanine. FIG. 5A shows that the NBD (WT) but not the mutant dose-dependently inhibited in vitro pull-down of [35S]-labeled IKK.beta. by GST-NEMO and [.sup.35S]-labeled NEMO by GST-IKK.beta.-(644-756). To test the ability of the NBD peptides to enter cells and inhibit the IKK.beta.-NEMO interaction, HeLa cells were incubated with the peptides for different time

periods and immunoprecipitated the **IKK complex** using anti-NEMO. In agreement with the in vitro data (FIG. 5A), wild-type but not mutant disrupted the formation of the endogenous **IKK complex** (FIG. 5B).

Detail Description Paragraph - DETX (164):

[0183] The importance of the present invention can be viewed on two levels. First, Applicants have identified the structural requirements for the association of NEMO with the **IKKs** and found that association with IKK.beta. is dependent on three amino acids (D738, W739 and W741) within the NBD. Furthermore, NEMO not only functions in the activation of IKK.beta. but it also has a critical role in suppressing the intrinsic, basal activity of the **IKK complex**. The second level of importance is the obvious clinical use for drugs targeting the NBD. Applicants have demonstrated that a cell-permeable peptide encompassing the NBD is able to not only inhibit TNF.alpha.-induced NF-.kappa.B activation but also reduce expression of E-selectin, an NF-.kappa.B-dependent target gene, in primary human endothelial cells. The NBD is only six amino acids long, and therefore it is within the ability of one skilled in the art to design peptido-mimetic compounds that disrupt the core **IKK complex**. Since the effect of disrupting the **complex** is to increase the basal activity of the **IKK,** treatment with an NBD-targeting compound can avoid issues of toxicity, e.g., due to hepatocyte apoptosis, that might arise from administering drugs that completely abolish the activity of NF-.kappa.B. Hence, identification of the NBD is a means for the development of novel anti-inflammatory drugs that prevent activating signals from reaching the **IKK complex,** yet maintain a low level of NF-.kappa.B activity and avoid potential toxic side-effects.

Detail Description Paragraph - DETX (176):

[0191] This data demonstrates that disruption of the core **IKK complex** by a cell permeable NBD peptide that inhibits NF-.kappa.B activation prevents RANKL-induced osteoclast differentiation indicating that drugs specifically targeting the NBD will be effective for the treatment of osteoporosis. As an extension of these in vitro studies, the same peptides can be analyzed for their effects on osteoporosis in vivo. Ovarectomized mice (Charles River Labs) that exhibit severe osteoporosis are treated with the NBD peptides and the effects on bone density over a timecourse of treatment determined.

Detail Description Paragraph - DETX (184):

[0195] In contrast to the lack of effects of the mutations described above on either NEMO binding or NF-.kappa.B activation, alanine substitution of the aspartic acid residue within the NBD (D738) prevented IKK.beta. from associating with NEMO. Furthermore, this substitution led to a 2- to 3-fold increase in the basal NF-KB-acivating ability of IKK.beta.. These results demonstrate a role for NEMO association in maintaining the basal activity of the **IKK complex**. Interestingly, treatment of HeLa cells with the cell-permeable NBD peptide also led to a modest increase in basal NF-.kappa.B

activity further supporting the concept that loss of NEMO association leads to increased basal **IKK** activity.

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TITLE: Prevention and treatment of amyloid-associated disorders

PUBLICATION-DATE: October 24, 2002

INVENTOR-INFORMATION:

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DATE FILED: June 14, 2002

RELATED-US-APPL-DATA:

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parent-patent 6428950 US

non-provisional-of-provisional 60109910 19981125 US

US-CL-CURRENT: 435/4, 435/7.21

ABSTRACT:

The present invention provides a method of assaying for and arresting, preventing and/or reversing the impairment of central and peripheral nervous system function comprising reducing .beta.-amyloid plaque burden by the administration of compounds that reduce apoE expression. The compounds used in the method of the invention may be: 1) inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase; 2) inhibitors of cholesterol biosynthesis; 3) inhibitors of protein isoprenylation, specifically geranylgeranylation; and/or 4) inhibitors of NF-.kappa.B activation or function. Assays for compounds with inhibit apoE expression from microglial cells are also disclosed.

---------- KWIC ---------

Detail Description Paragraph - DETX (42):

[0071] Important modulators of NF-.kappa.B activation are the inhibitor proteins I.kappa.B.sub..alpha. and I.kappa.B.sub..beta.. (referred to herein as I.kappa.B), which associate with, and thereby, inactivate, NF-.kappa.B in vivo. Activation and nuclear translocation of NF-.kappa.B occurs following signal-induced phosphorylation of I.kappa.B, which leads to proteolysis via the ubiquitin pathway. This pathway includes a cascade of atypical protein kinases that catalyze the ubiquination of I.kappa.B, the I.kappa.B kinase (**IKK) complex** (FIG. 4).

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020151021 A1

TITLE: Stimulus-inducible protein kinase complex and methods of use therefor

PUBLICATION-DATE: October 17, 2002

INVENTOR-INFORMATION:

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DATE FILED: April 27, 2001

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child 08910820 19970813 US

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US-CL-CURRENT: 435/194, 435/252.3 , 435/254.2 , 435/320.1 , 435/325 , 435/348 , 435/69.1

ABSTRACT:

Compositions and methods are provided for treating NF-.kappa.B-related conditions. In particular, the invention provides a stimulus-inducible IKK signalsome, and components and variants thereof. An IKK signalsome or component thereof may be used, for example, to identify antibodies and other modulating agents that inhibit or activate signal transduction via the NF-.kappa.B cascade. IKK signalsome, components thereof and/or modulating agents may also be used for the treatment of diseases associated with NF-.kappa.B activation.

CROSS-REFERENCE TO PRIOR APPLICATION

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 08/697,393, filed Aug. 26, 1996.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (12):

[0033] FIGS. 8A-8C are autoradiograms depicting the results of immunoblot analyses. In FIG. 8A, the upper panel presents a time course for the induction of signalsome activity. Anti MKP-1 immune precipitates from extracts of HeLa S3 cells stimulated with TNF.alpha. (20 ng/ml) for the indicated times, were assayed for **IKK** signalsome activity by standard immune **complex** kinase assays. 4 .mu.g of either GST I.kappa.B.alpha. 1-54 WT (wildtype) or the GST I.kappa.B.alpha. 1-54 S32/36 to T mutant (S>T) were used as the substrates. In the lower panel, HeLa cell extracts prepared as described in the upper panel were examined by western blot analysis for I.kappa.B.alpha. degradation. I.kappa.B.alpha. supershifting phosphorylation can be seen after 3 and 5 minutes of stimulation followed by the disappearance of I.kappa.B.alpha..

Brief Description of Drawings Paragraph - DRTX (14):

[0035] FIG. 8C illustrates the ability of **IKK** signalsome to specifically phosphorylate serines 32 and 36 of the I.kappa.B.alpha. holoprotein in the context of a RelA:I.kappa.B.alpha. **complex**. Anti-MKP-1 immunoprecipitates from cell extracts of HeLa S3 cells stimulated with TNF.alpha. (20 ng/ml, 7 min) were examined for their ability to phosphorylate baculoviral expressed RelA:I.kappa.B.alpha. **complex** containing either the I.kappa.B.alpha. WT (lane 3) or I.kappa.B.alpha. S32/36 to A mutant (lane 4) holoprotein. The specific substrates used are indicated on the top. Positions of the phosphorylated substrates are indicated by arrows to the left of the panel.

Brief Description of Drawings Paragraph - DRTX (17):

[0038] FIG. 10 is an autoradiogram showing the results of a western blot analysis of the level of ubiquitin within a stimulus-inducible IkB kinase **complex**. Lane 1 shows the detection of 100 ng ubiquitin, Lane 2 shows 10 ng ubiquitin and Lane 3 shows 3.4 .mu.g of **IKK** signalsome purified through the phenyl superose step (sufficient quantities for 10 kinase reactions). The position of ubiquitin is shown by the arrow on the left.

Detail Description Paragraph - DETX (2):

[0047] As noted above, the present invention is generally directed to compositions and methods for modulating (i.e., stimulating or inhibiting) signal transduction leading to NF-.kappa.B activation. In particular, the present invention is directed to compositions comprising an I.kappa.B kinase (**IKK**) signalsome (also referred to herein as a "stimulus-inducible I.kappa.B kinase **complex**" or "I.kappa.B kinase **complex**") that is capable of stimulus-dependent phosphorylation of I.kappa.B.alpha. and I.kappa.B.beta. on the two N-terminal serine residues critical for the subsequent ubiquitination

and degradation in vivo. Such stimulus-dependent phosphorylation may be achieved without the addition of exogenous cofactors. In particular, an **IKK** signalsome specifically phosphorylates I.kappa.B.alpha. (SEQ ID NO:1) at residues S32 and S36 and phosphorylates I.kappa.B.beta. (SEQ ID NO:2) at residues S19 and S23. The present invention also encompasses compositions that contain one or more components of such an **IKK** signalsome, or variants of such components. Preferred components, referred to herein as "**IKK** signalsome kinases" "I.kappa.B kinases" or **IKKs**) are kinases that, when incorporated into an **IKK** signalsome, are capable of phosphorylating I.kappa.B.alpha. at S32 and S36. Particularly preferred components are **IKK-1** (SEQ ID NO:10) and **IKK-2** (SEQ ID NO:9).

Detail Description Paragraph - DETX (4):

[0049] An **IKK** signalsome has several distinctive properties. Such a **complex** is stable (i.e., its components remain associated following purification as described herein) and has a high-molecular weight (about 500-700 kD, as determined by gel filtration chromatography). As shown in FIGS. 3 (A and B) and 4 (A and B), I.kappa.B kinase activity of an **IKK** signalsome is "stimulus-inducible" in that it is stimulated by TNF.alpha. (i.e., treatment of cells with TNF.alpha. results in increased I.kappa.B kinase activity and I.kappa.B degradation) and/or by one or more other inducers of NF-.kappa.B, such as IL-1, LPS, TPA, UV irradiation, antigens, viral proteins and stress-inducing agents. The kinetics of stimulation by TNF.alpha. correspond to those found in vivo. I.kappa.B kinase activity of an **IKK** signalsome is also specific for S32 and S36 of I.kappa.B.alpha.. As shown in FIGS. 5 (A and B) and 6 (A and B), an **IKK** signalsome is capable of phosphorylating a polypeptide having the N-terminal sequence of I.kappa.B.alpha. (GST-I.kappa.B.alpha.1-54; SEQ ID NO:3), but such phosphorylation cannot be detected in an I.kappa.B.alpha. derivative containing threonine substitutions at positions 32 and 36. In addition, I.kappa.B kinase activity is strongly inhibited by a doubly phosphorylated I.kappa.B.alpha. peptide (i.e., phosphorylated at S32 and S36), but not by an unrelated c-fos phosphopeptide that contains a single phosphothreonine. A further characteristic of an **IKK** signalsome is its ability to phosphorylate a substrate in vitro in a standard kinase buffer, without the addition of exogenous cofactors. Free ubiquitin is not detectable in preparations of **IKK** signalsome (see FIG. 10), even at very long exposures. Accordingly an **IKK** signalsome differs from the ubiquitin-dependent I.kappa.B.alpha. kinase activity described by Chen et al., Cell 84:853-62, 1996.

Detail Description Paragraph - DETX (5):

[0050] An **IKK** signalsome may be immunoprecipitated by antibodies raised against MKP-1 (MAP kinase phosphatase-1; Santa Cruz Biotechnology, Inc., Santa Cruz, Calif. #SC-1102), and its activity detected using an in vitro I.kappa.B.alpha. kinase assay. However, as discussed further below, MKP-1 does not appear to be a component of I.kappa.B kinase **complex**. The substrate specificity of the immunoprecipitated **IKK** signalsome is maintained (i.e., there is strong phosphorylation of wildtype GST-I.kappa.B.alpha. 1-54 (SEQ ID NO:3)

and GST-I.kappa.B.beta. 1-44 (SEQ ID NO:4), and substantially no detectable phosphorylation of GST-I.kappa.B.alpha. 1-54 in which serines 32 and 36 are replaced by threonines (GST- I.kappa.B.alpha. S32/36 to T; SEQ ID NO:5) or GST-I.kappa.B.beta. 1-44 in which serines 19 and 23 are replaced by alanines (GST-I.kappa.B.beta. 1-44 S19/23 to A; SEQ ID NO:6)).

Detail Description Paragraph - DETX (8):

[0053] Throughout the fractionation, an in vitro kinase assay may be used to monitor the I.kappa.B kinase activity of the **IKK** signalsome. In such an assay, the ability of a fraction to phosphorylate an appropriate substrate (such as I.kappa.B.alpha. (SEQ ID NO:1) or a derivative or variant thereof) is evaluated by any of a variety of means that will be apparent to those of ordinary skill in the art. For example, a substrate may be combined with a chromatographic fraction in a protein kinase buffer containing .sup.32P .gamma.-ATP, phosphatase inhibitors and protease inhibitors. The mixture may be incubated for 30 minutes at 30.degree. C. The reaction may then be stopped by the addition of SDS sample buffer and analyzed using SDS-PAGE with subsequent autoradiography. Suitable substrates include full length I.kappa.B.alpha. (SEQ ID NO:1), polypeptides comprising the N-terminal 54 amino acids of I.kappa.B.alpha., full length I.kappa.B.beta. (SEQ ID NO:2) and polypeptides comprising the N-terminal 44 amino acids of I.kappa.B.beta.. Any of these substrates may be used with or without an N-terminal tag. One suitable substrate is a protein containing residues 1-54 of I.kappa.B.alpha. and an N-terminal GST tag (referred to herein as GST-I.kappa.B.alpha. 1-54; SEQ ID NO:3). To evaluate the specificity of an I.kappa.B kinase **complex,** I.kappa.B.alpha. mutants containing threonine or alanine residues at positions 32 and 36, and/or other modifications, may be employed.

Detail Description Paragraph - DETX (9):

[0054] Alternatively, an **IKK** signalsome may be prepared from its components which are also encompassed by the present invention. Such components may be produced using well known recombinant techniques, as described in greater detail below. Components of an **IKK** signalsome may be native, or may be variants of a native component (ie., a component sequence may differ from the native sequence in one or more substitutions and/or modifications, provided that the ability of a **complex** comprising the component variant to specifically phosphorylate I.kappa.B.alpha. is not substantially diminished). Substitutions and/or modifications may generally be made in non-critical and/or critical regions of the native protein. Variants may generally comprise residues of L-amino acids, D-amino acids, or any combination thereof. Amino acids may be naturally-occurring or may be non-natural, provided that at least one amino group and at least one carboxyl group are present in the molecule; .alpha.- and .beta.-amino acids are generally preferred. A variant may also contain one or more rare amino acids (such as 4-hydroxyproline or hydroxylysine), organic acids or amides and/or derivatives of common amino acids, such as amino acids having the C-terminal carboxylate esterified (e.g., benzyl, methyl or ethyl ester) or amidated and/or having modifications of the N-terminal amino group (e.g., acetylation or alkoxycarbonylation), with or without any of a wide variety of side-chain modifications and/or substitutions

(e.g., methylation, benzylation, t-butylation, tosylation, alkoxycarbonylation, and the like). Component variants may also, or alternatively, contain other modifications, including the deletion or addition of amino acids that have minimal influence on the activity of the polypeptide. In particular, variants may contain additional amino acid sequences at the amino and/or carboxy termini. Such sequences may be used, for example, to facilitate purification or detection of the component polypeptide. In general, the effect of one or more substitutions and/or modifications may be evaluated using the representative assays provided herein.

Detail Description Paragraph - DETX (11):

[0056] Alternatively, partial sequences of the components may be obtained using standard biochemical purification and microsequencing techniques. For example, purified **complex** as described above may be run on an SDS-PAGE gel and individual bands may be isolated and subjected to protein microsequencing. DNA sequences encoding components may then be prepared by amplification from a suitable human cDNA library, using polymerase chain reaction (PCR) and methods well known to those of ordinary skill in the art. For example, an adapter-ligated cDNA library prepared from a cell line or tissue that expresses **IKK** signalsome (such as HeLa or Jurkat cells) may be screened using a degenerate 5' specific forward primer and an adapter-specific primer. Degenerate oligonucleotides may also be used to screen a cDNA library, using methods well known to those of ordinary skill in the art. In addition, known proteins may be identified via Western blot analysis using specific antibodies.

Detail Description Paragraph - DETX (13):

[0058] Particularly preferred components of **IKK** signalsome are I.kappa.B kinases. An I.kappa.B kinase may be identified based upon its ability to phosphorylate one or more I.kappa.B proteins, which may be readily determined using the representative kinase assays described herein. In general, an I.kappa.B kinase is incorporated into an **IKK** signalsome, as described herein, prior to performing such assays, since an I.kappa.B kinase that is not **complex**-associated may not display the same phosphorylation activity as **complex**-associated I.kappa.B kinase. As noted above, an I.kappa.B kinase within an **IKK** signalsome specifically phosphorylates I.kappa.B.alpha. at residues S32 and S36, and phosphorylates I.kappa.B.beta. at residues 19 and 23, in response to specific stimuli.

Detail Description Paragraph - DETX (22):

[0067] In one aspect of the present invention, an **IKK** signalsome and/or one or more components thereof may be used to identify modulating agents, which may be antibodies (e.g., monoclonal), polynucleotides or other drugs, that inhibit or stimulate signal transduction via the NF-.kappa.B cascade. Modulation includes the suppression or enhancement of NF-.kappa.B activity. Modulation may also include suppression or enhancement of I.kappa.B phosphorylation or the stimulation or inhibition of the ability of activated (i.e., phosphorylated)

IKK signalsome to phosphorylate a substrate. Compositions that inhibit NF-.kappa.B activity by inhibiting I.kappa.B phosphorylation may include one or more agents that inhibit or block I.kappa.B.alpha. kinase activity, such as an antibody that neutralizes **IKK** signalsome, a competing peptide that represents the substrate binding domain of I.kappa.B kinase or a phosphorylation motif of I.kappa.B, an antisense polynucleotide or ribozyme that interferes with transcription and/or translation of I.kappa.B kinase, a molecule that inactivates **IKK** signalsome by binding to the **complex,** a molecule that binds to I.kappa.B and prevents phosphorylation by **IKK** signalsome or a molecule that prevents transfer of phosphate groups from the kinase to the substrate. Within certain embodiments, a modulating agent inhibits or enhances the expression or activity of **IKK-1** and/or **IKK-2**.

Detail Description Paragraph - DETX (25):

[0070] In another aspect of the present invention, **IKK** signalsome or I.kappa.B kinase may be used for phosphorylating an I.kappa.B such as I.kappa.B.alpha. (or a derivative or variant thereof) so as to render it a target for ubiquitination and subsequent degradation. I.kappa.B may be phosphorylated in vitro by incubating **IKK** signalsome or I.kappa.B kinase with I.kappa.B in a suitable buffer for 30 minutes at 30.degree. C. In general, about 0.01 .mu.g to about 9 .mu.g of I.kappa.B kinase **complex** is sufficient to phosphorylate from about 0.5 .mu.g to about 2 .mu.g of I.kappa.B. Phosphorylated substrate may then be purified by binding to GSH-sepharose and washing. The extent of substrate phosphorylation may generally be monitored by adding [.gamma.-.sup.32P]ATP to a test aliquot, and evaluating the level of substrate phosphorylation as described herein.

Detail Description Paragraph - DETX (34):

[0079] In another aspect, the present invention provides methods for detecting the level of stimulus-inducible I.kappa.B kinase activity in a sample. The level of I.kappa.B kinase activity may generally be determined via an immunokinase assay, in which **IKK** signalsome is first immunoprecipitated with an antibody that binds to the **complex**. The immunoprecipitated material is then subjected to a kinase assay as described herein. Substrate specificity may be further evaluated as described herein to distinguish the activity of a stimulus-inducible I.kappa.B kinase **complex** from other kinase activities.

Detail Description Paragraph - DETX (39):

[0084] Monoclonal antibodies specific for an **IKK** signalsome or a component thereof may be prepared, for example, using the technique of Kohler and Milstein, Eur. J. Immunol. 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (i.e., reactivity with the **complex** and/or component of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a

myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Detail Description Paragraph - DETX (48):

[0090] This example illustrates the recruitment of NF.kappa.B into a protein **complex (the IKK** signalsome) containing I.kappa.B kinase and other signaling proteins.

Detail Description Paragraph - DETX (50):

[0092] As shown in FIG. 1A, I.kappa.B.alpha. in cell extracts from unstimulated cells eluted with an apparent molecular weight of .about.300 kDa (lanes 5-7), consistent with the chromatographic properties of the inactive NF.kappa.B-I.kappa.B **complex** (Baeuerle and Baltimore, Genes Dev. 3:1689-98, 1989). In contrast, phosphorylated I.kappa.B.alpha. (from cells stimulated for periods too short to permit complete degradation of the protein) migrated at .about.600 kDa on the same chromatography columns (lanes 2, 3). This difference in migration was specific for I.kappa.B, since analysis of the same fractions indicated that the Jun N-terminal kinases JNK1 and JNK2 migrated with low apparent molecular weight and showed no difference in chromatographic behavior between stimulated and unstimulated cells. Stimulation-dependent recruitment of I.kappa.B into this larger protein **complex** corresponded with the appearance of phosphorylated I.kappa.B, suggesting that the **complex** contained the specific I.kappa.B kinases that mediate I.kappa.B phosphorylation. These results demonstrate that that NF.kappa.B activation involves recruitment into a protein **complex (the IKK** signalsome) containing I.kappa.B kinase and other signaling proteins.

Detail Description Paragraph - DETX (58):

[0096] This Example illustrates an alternate preparation of an **IKK** signalsome, and the characterization of the **complex**.

Detail Description Paragraph - DETX (84):

[0120] Of a large panel of antibodies tested, one of three anti-MKP-1 antibodies efficiently co-immunoprecipitated an inducible I.kappa.B kinase activity from HeLa cells as well as primary human umbilical vein endothelial cells (HUVEC). The co-immunoprecipitated kinase (**IKK** signalsome kinase) was inactive in unstimulated HeLa cells, but was rapidly activated within minutes

of TNF.alpha. stimulation (FIG. 8A, top panel). The **IKK** signalsome kinase did not phosphorylate a mutant GST-I.kappa.B.alpha. protein in which serine residues 32 and 36 had been mutated to threonine (FIG. 8A top panel, even-numbered lanes). Activation of the signalsome kinase was maximal at 5 minutes and declined thereafter, a time course consistent with the time course of I.kappa.B.alpha. phosphorylation and degradation under the same conditions (FIG. 8A, bottom panel). As expected, the signalsome I.kappa.B kinase was also activated by stimulation of cells with IL-1 or PMA (FIG. 8B, lanes 1-4); moreover, its activity was inhibited in cells treated with TPCK, a known inhibitor of NF.kappa.B activation (FIG. 8B, lane 7). Additionally, the **IKK** signalsome kinase specifically phosphorylated full-length wild-type I.kappa.B.alpha., but not a mutant I.kappa.B.alpha. bearing the serine 32, 36 to alanine mutations, in the context of a physiological RelA-I.kappa.B.alpha. **complex** (FIG. 8C, lanes 3, 4). Together these results indicate that the anti-MKP-1 antibody co-immunoprecipitated the **IKK** signalsome. The signalsome-associated I.kappa.B kinase met all the criteria expected of the authentic I.kappa.B kinase and had no detectable I.kappa.B.alpha. C-terminal kinase activity.

Detail Description Paragraph - DETX (93):

[0127] This example illustrates the absence of detectable free ubiquitin with a **IKK** signalsome prepared as in Example 3. Standard western blot procedures were performed (Amersham Life Science protocol, Arlington Heights, Ill.). 100 ng ubiquitin, 10 ng ubiquitin and 20 .mu.l purified IkB.alpha. kinase **complex** was subjected to 16% Tricine SDS-PAGE (Novex, San Diego, Calif.), transferred to Hybond ECL Nitrocellulose membrane (Amersham Life Science, Arlington Heights, Ill.), and probed with antibodies directed against ubiquitin (MAB1510; Chemicon, Temecula, Calif.). The results are shown in FIG. 10. Free ubiquitin could not be detected in the purified IkB.alpha. kinase preparation (even at very long exposures). The complexes described herein do not require addition of endogenous ubiquitin to detect IkB.alpha. kinase activity, nor is free ubiquitin a component in the purified IkB.alpha. kinase preparations of the present invention.

Detail Description Paragraph - DETX (107):

[0139] Both **IKK-1 and IKK-2** kinases were active when expressed in wheat germ extracts, since they were capable of autophosphorylation, but they were inactive with respect to phosphorylation of I.kappa.B substrates. Since both autophosphorylation and substrate phosphorylation were intact in rabbit reticulocyte lysates, there appeared to be a direct correlation between the association of **IKK-1 and IKK-2** into a higher order protein **complex** and the presence of specific I.kappa.B kinase activity in **IKK-1 and IKK-2** immunoprecipitates. This higher order **complex** is most likely the **IKK** signalsome itself. Indeed, immunoprecipitation of rabbit reticulocyte lysates with anti-MKP-1 antibody pulls down a low level of active I.kappa.B kinase activity characteristic of the **IKK** signalsome.

Detail Description Paragraph - DETX (108):

[0140] It is clear that the **IKK** signalsome contains multiple protein components in addition to **IKK-1 and IKK-2** (FIG. 11B). Some of these may be upstream kinases such as MEKK-1 (Chen et al., Cell 84:853-62, 1996) or NIK (Malinin, et al., Nature 385:540-44, 1997); others may be adapter proteins that mediate the **IKK**-1:**IKK**-2 interaction. Indeed MEKK-1 copurifies with **IKK** signalsome activity (FIG. 1C), and two other signalsome proteins have been functionally identified. The protein crossreactive with anti-MKP-1 is an intrinsic component of the **IKK** signalsome kinases, since the I.kappa.B kinase activity coprecipitated with this antibody is stable to washes with 2-4 M urea Moreover, both **IKK-1** immunoprecipitates and MKP-1 immunoprecipitates containing the **IKK** signalsome (FIG. 8C) contain an inducible RelA kinase whose kinetics of activation parallel those of the I.kappa.B kinase in the same immunoprecipitates. Another strong candidate for a protein in the signalsome **complex** is the E3 ubiquitin ligase that transfers multiubiquitin chains to phosphorylated I.kappa.B (Hershko et al., Annu. Rev. Biochem. 61:761-807,1992).

Detail Description Paragraph - DETX (109):

[0141] These results indicate that **IKK-1 and IKK-2** are functional kinases within the **IKK** signalsome, which mediate I.kappa.B phosphorylation and NF.kappa.B activation. Appropriate regulation of **IKK-1 and IKK-2** may require their assembly into a higher order protein **complex,** which may be a heterodimer facilitated by adapter proteins, the complete **IKK** signalsome, or some intermediate subcomplex that contains both **IKK-1 and IKK-2**.

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TITLE: Composition and method for smoke detoxification

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INVENTOR-INFORMATION:

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APPL-NO: 10/ 058299

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RELATED-US-APPL-DATA:

non-provisional-of-provisional 60267428 20010209 US

US-CL-CURRENT: 424/729, 424/756

ABSTRACT:

A method is provided for effecting smoke detoxification in a human by using a composition that is made of effective amounts of supercritical extract and hydroalcoholic extract of turmeric.

[0001] This application claims the benefit of U.S. Provisional Application No. 60/267,428, filed Feb. 9, 2001.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (5):

[0004] Turmeric has been found to be effective in inhibiting the formation and excretion of urinary mutagens in smokers. (See P. Kalpagam, T. C. Raghuram, T. P. Krishna and K. Krishnaswamy, "Effect of Turmeric on Urinary Mutagens in Smokers", Mutagenesis, vol. 7, no. 2, pp. 107-109 (1992) (stating that tobacco mutagens may be detoxified by the active principle curcumin)). Turmeric has also been found to be an effective anti-mutagen and may be useful in chemoprevention. Articles discussing turmeric and/or curcumin include: Krishnaswamy, K., and Raghuramulu, N., Bioactive Phytochemicals with Emphasis on Dietary Practices, Indian J Med Res 108, November 1998, pp. 167-181; Deshpande, S. S., Ingle, A. D., and Maru, G. B., Inhibitory Effects of Curcumin-Free Aqueous Turmeric Extract on Benzo[alpha]pyrene-Induced

Forestomach Papillomas in Mice, Cancer Letters, 118 (1997) 79-85; Srimal R. C., Turmeric: A Brief Review of Medicinal Properties, Fitoterapia, Vol. LXVIII, No. 6, 1997, pp. 483-493; Arbiser, J. L., Klauber, N., Rohan, R., van Leeuwen, R., Huang, M. T., Fisher, C., Flynn, E., Byers, H. R., Curcumin is an In Vivo Inhibitor of Angiogenesis, Mol Med (June 1998), 4(6):376-83; Plummer, S. M., Holloway, K. A., Manson, M. M., Munks, R. J., Kaptein, A., Farrow, S., and Howells, L., Inhibition of Cyclo-Oxygenase 2 Expression in Colon Cells by the Chemopreventive Agent Curcumin Involves Inhibition of NF-kappaB activation Via the NIK/**IKK** Signaling **Complex,** Oncogene (Oct. 28, 1999), 18(44):6013-20; Singhal, S. S., Awasthi, S., Pandya, U., Piper, J. T., Saini, M. K., Cheng, J. Z., and Awasthi, Y. C., The Effect of Curcumin on Glutathione-Linked Enzymes in K562 Human Leukemia Cells, Toxicol Lett, Sep. 20, 1999, 109(1-2):87-95; Kang, B. Y., Song, Y. J., Kim, K. M., Choe, Y. K., Hwang, S. Y., Kim, T. S., Curcumin Inhibits Th1 Cytokine Profile in CD4+ T Cells By Suppressing Interleukin-12 Production in Macrophages, Br J Pharmacol, September 1999, 128(2):380-4.

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TITLE: Nod2 nucleic acids and proteins

PUBLICATION-DATE: September 12, 2002

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/183, 435/320.1 , 435/325 , 435/410 , 435/69.1 , 536/23.2 , 800/278 , 800/8

ABSTRACT:

The present invention relates to intracellular signalling molecules, in particular the Nod2 protein and nucleic acids encoding the Nod2 protein. The present invention provides isolated nucleotide sequence encoding Nod2, isolated Nod2 peptides, antibodies that specifically bind Nod2, methods for the detection of Nod2, and methods for screening compounds for the ability to alter Nod2 associated signal transduction.

[0001] This application claims priority to U.S. provisional patent application serial No. 60/244,289, which is herein incorporated by reference in its entirety. This patent application was supported in part by grant CA-64556 from the National Institutes of Health. The government has certain rights in the invention.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (12):

[0036] FIG. 5 shows that Nod2 Acts through the **IKK complex** to activate NF-KB.

Detail Description Paragraph - DETX (5):

[0056] Nod2 is the first molecule known to contain two CARDS. The molecular basis underlying the requirement of both CARDs of Nod2 for RICK binding remains unclear. The present invention is not limited to any particular mechanism of action. Indeed, an understanding of the mechanism of action is not necessary to practice the present invention. Nevertheless, it is contemplated that the presence of both CARDs may enhance the affinity for the CARD of RICK. Another possibility is that upon an initial interaction involving a CARD of Nod2 and the CARD of RICK, Nod2 may undergo a conformational change that allows the second CARD to associate with high affinity to RICK. The intermediate region of RICK associates with IKK.gamma. (Inohara et al., [2000], supra), providing a direct link between Nod1/Nod2 and the **IKK complex**. Consistent with this model, NF-.kappa.B activation induced by Nod2 as well as that induced by Nod I required IKK.gamma. and was inhibited by dominant negative forms of IKK.gamma., IKK.alpha. and IKK.beta.. The functional role for the LRRs of Nod1 and Nod2 remains unclear. The LRR is a repeated protein-protein interaction module that is presumably involved in the activation of Nod1 and Nod2 by upstream signals. In the case of plant NBD/LRR-containing R proteins, their LRRs appear to be important for the recognition of pathogen components and their N-terminal domains appear to mediate a signaling cascade that regulates gene expression (Parniske et al., supra, Dixon et al., supra). Because both Nod1 and Nod2 activate NF-.kappa.B, their LRRs may act to recognize a different set of intracellular stimuli that mediate Nod1 and Nod2 oligomerization and association with RICK. Because Nod2 is expressed primarily in monocytes, Nod2 might serve as an intracellular receptor that transduces signals in the monocyte/macrophage that lead to activation of NF-.kappa.B and transcription of regulatory genes.

Detail Description Paragraph - DETX (328):

[0373] This example demonstrates that NF-.kappa.B activation induced by Nod2 requires IKK.gamma. and is inhibited by dominant negative forms of **IKKs** and RICK. A main pathway of NF-.kappa.B activation is mediated by I.kappa.B kinases (**IKKS**) resulting in I.kappa.B phosporylation and release of cytoplasmic NF-.kappa.B (Karin, J. Biol. Chem. 274: 27339-27342 [1999]). To determine whether Nod2 activates an **IKK**-dependent pathway, Nod2 was co-expressed with mutant forms of IKK.alpha., IKK.beta., and I.kappa.B that have been shown to act as dominant inhibitors of their corresponding endogenous counterparts and/or the **IKK complex** (Karin, supra). In addition, a truncated mutant of IKK.gamma./Nemo (residues 134-419) was used that is defective in IKK.alpha. and IKK.beta. binding and acts as an inhibitor of NF-.kappa.B activation induced by RIP and RICK (Inohara et al., [2000], supra). The NF-.kappa.B activity induced by Nod2 as well as that induced by TNF.alpha. stimulation were greatly inhibited by mutant IKK.alpha., IKK.beta., IKK.gamma., and I.kappa.B.alpha. (FIG. 5A). Because RICK has been shown to serve as a downstream target of Nod1 (Bertin et al., supra, Inohara et al., [1999] supra, Inohara et al., [2000], supra), a truncated form of RICK containing its CARD (residues 406-540) that acts as a dominant inhibitor of Nod1 activity (Bertin et al., supra) was used to test whether NF-.kappa.B activation induced by Nod2 is similarly inhibited by this RICK mutant. NF-.kappa.B activation induced by

Nod2 was inhibited by mutant RICK but not by a mutant form of RIP that expresses its death effector domain (FIG. 5A). The inhibition by the CARD of RICK was specific in that it did not interfere with ability of TNF.alpha. to induce NF-.kappa.B, an activity that was inhibited by the RIP mutant (FIG. 5A). To verify that Nod2 acts upstream of the **IKK complex** to activate NF-.kappa.B, we tested the ability of Nod2 to activate NF-.kappa.B in parental Rat1 fibroblasts and 5R cells, a Rat1 derivative cell line that is defective in IKK.gamma., an essential subunit of the **IKKs** (Yamaoka et al., supra). Nod2, as well as Nod1 and TNFoc, induced NF-KB activity in parental Rat1 cells but not in IKK.gamma.-deficient 5R cells (FIG. 5B). As a control, expression of IKK.beta., which functions downstream of IKK.gamma., induced NF-.kappa.B activation in both Rat1 and 5R cell lines (FIG. 5B). These results indicate that Nod2 acts through IKK.gamma./**IKK**/IKK.beta. to activate NF-.kappa.B.

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TITLE: Compositions and methods for production cell culture

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INVENTOR-INFORMATION:

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ABSTRACT:

The invention provides improved methods of recombinant protein production in cell culture. More specifically, the invention relates to the activation of NF-kappa-B transcription factor complex in cells so as to improve production characteristics.

RELATED APPLICATION DATA

[0001] This application claims the benefit of provisional U.S. application No. 60/270,943, filed Feb. 22, 2001, the entire disclosure of which is incorporated by reference herein.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (22):

[0018] Still another way of activating NF-kappa-B that is within the scope of the invention is to increase the expression or activity of the **IKK** family members which, when activated, target for degradation the IKB family members. As noted above, **IKK** family members include but are not limited to **IKK-1 and IKK-2**. Also encompassed within the invention is the activation of the NF-kappa-B transcription factor **complex** by any combination of the above.

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TITLE: TRAF-3 deletion isoforms and uses thereof

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INVENTOR-INFORMATION:

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RELATED-US-APPL-DATA:

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child PCT/US00/06503 20000310 US

parent continuation-in-part-of 09268544 19990311 US PENDING

US-CL-CURRENT: 435/7.21, 435/183 , 435/320.1 , 435/325 , 530/350 , 536/23.2

ABSTRACT:

The present invention provides an isolated TRAF-3 deletion isoform encoded by the nucleic acid sequence (SEQ ID NO: 1) shown in FIG. 15 (deletion isoform &Dgr; 130 nucleic acid). One embodiment of the present invention is an isolated TRAF-3 protein which comprises the sequence in FIG. 16 (SEQ ID NO: 2) (&Dgr. 130 protein). Another embodiment of the present invention is an isolated TRAF-3 deletion isoform encoded by the nucleic acid sequence (SEQ ID NO: 3) shown in FIG. 17 (deletion isoform &Dgr-221 nucleic acid). A further embodiment of the present invention is an isolated TRAF-3 deletion isoform protein which comprises the sequence in FIG. 18 (SEQ ID NO: 4) (deletion isoform &Dgr.221 protein). The invention also provides a method for identifying an agent that inhibits CD40-mediated cellular signaling in a cell which comprises: (a) contacting the cell with an agent under conditions wherein CD40-mediated cell activation occurs, and (b) determining whether CD40-mediated signaling is inhibited in the cell in the presence of the agent so as to identify whether the agent inhibits CD40-mediated cellular signaling.

---------- KWIC ---------

Detail Description Paragraph - DETX (241):

[0260] The finding that TRAF-3 .DELTA.130aa isoform induced the highest NF-.kappa.B activation after over-expression in BJAB cells, is interesting in light of the fact that .DELTA.130aa encoding mRNA was undetectable in any of the resting lymphoma lines. Since TRAF-3 .DELTA.130aa encoding mRNA was cloned from Jurkat D1.1 (van Eyndhoven et al., 1999), these data suggest that .DELTA.130aa is expressed under certain circumstances, and may be both potent and closely regulated. It remains unclear how TRAF-3 over-expression models receptor-induced signaling, but the possibility that receptor aggregation (by ligand) liberates TRAF-3 from receptor tails is suggested by consideration that high local concentrations of receptor tails and TRAF-3 homotrimers would favor TRAF-3 homotrimers either binding three or no receptor tails (Pullen et al., 1999). The downstream events in TRAF-3 signaling may be cell type restricted since four TRAF-3 splice-variants which induce NF-.kappa.B activation in 293 cells (van Eyndhoven et al., 1999) failed to induce NF-.kappa.B activation in BJAB cells. The basis of this cell-type restricted signaling are not yet understood, but may relate to differences in expression of TRAF binding kinases that stimulate the **IKK complex,** such as NIK, MEKK1, GCK, and GCKR (Malinin et al., 1997; Song et al., 1997; Baud et al., 1999; Yuasa et al., 1998; Shi and Kehrl, 1997; Chin et al., 1999). TRAF-2 signaling also appeared to be cell-type restricted since over-expression of TRAF-2 failed to induce NF-.kappa.B activation in BJAB whereas TRAF-5 was active.

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TITLE: Variants of protein kinases

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INVENTOR-INFORMATION:

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RELATED-US-APPL-DATA:

child 09771161 A1 20010126

parent continuation-in-part-of 09724676 20001128 US PENDING

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
IL	136776	2000IL-136776	June 15, 2000
IL	135619	2000IL-135619	May 12, 2000

US-CL-CURRENT: 435/6, 530/350 , 536/23.1

ABSTRACT:

The present invention concerns nucleic acid sequences and amino acid sequences of dominant negative variants of kinases, i.e. of sequences which inhibit activity of kinases in a dominant manner. The invention also concerns pharmaceutical compositions and detection methods using these sequences.

---------- KWIC ---------

Summary of Invention - Table CWU - BSTL (4):

mutation locus) 43 28704 20386 STE20-related AB013385 Dementia; protein kinase parkinsonism (only potentially because of mutation locus) 44 28720 20399 STE20-related AB013385 Dementia; protein kinase parkinsonism (only potentially because of mutation locus) 45 31631492 22560 receptor tyrosine TYROSINE- M83941 HEK is a member of Wicks et al. 42 kinase (HEK) PROTEIN the EPH/ELK Proc. Natl. Acad. KINASE family of receptor Sci. U.S.A. 89 (5),

RECEPTOR tyrosine kinases. 1611-1615 (1992) ETK1 46 31672 22601 ser-thr protein U59305 Protein kinase similar to DMPK Zhao et al. kinase PK428 related to the (Myotonic dystrophy) Submitted myotonic dystrophy (MAY-1996) to the Protein kinase EMBL/GenBank/ family DDBJ databases. 47 30095 21393 serine threonine SERINE/ AF035625 May be a member Mutation causes the Nezu J.; kinase 11 THREONINE- of a yet unidentified Peutz-Jeghers Submitted (STK11) PROTEIN signaling pathway syndrome (PJS), a (AUG-1996) to the KINASE and it may act as rare hereditary EMBL/GenBank/ LKB1 a tumor-suppressor. disease in which DDBJ databases. there is predisposition to benign and malignant tumors of many organ systems. Pjs is an autosomal-dominant disorder characterized by melanocytic macules of the lips, multiple gastrointestinal hamartomatous polyps and an increased risk for various neoplasms, including gastrointestinal cancer. Many splice variants are known. 48 30096 21394 serine threonine SERINE/ AF035625 May be a member Mutation causes the Nezu J.; kinase 11 THREONINE- of a yet unidentified Peutz-Jeghers Submitted (STK11) PROTEIN signaling pathway syndrome (PJS), a (AUG-1996) to the KINASE and it may act as rare hereditary EMBL/GenBank/ LKB1 a tumor-suppressor. disease in which DDBJ databases. there is predisposition to benign and malignant tumors of many organ systems. Pjs is an autosomal-dominant disorder characterized by melanocytic macules of the lips, multiple gastrointestinal hamartomatous polyps and an increased risk for various neoplasms, including gastrointestinal cancer. Many splice variants are known. 49 28913 20573 RIP-like kinase AF156884 An adaptor proteins Yu, et al. (RIP3) that contain death Curr. Biol. 9 (10), domains. RIP3 539-542 (1999) appears to function as an intermediary in tnfalpha-induced apoptosis. 50 28768 20439 IkB kinase-b AF080158 Serine/threonine The activity of the Hu et al. (**IKK**-beta) kinases which has kinase prevents the Gene 222 (1), 31-40 been identified as pathogenesis of the (1998) ikappab kinase inflammatory which is essential response. for ikappab phosphorylation and NF-kappab activation 51 30947 22055 mRNA for LIMK-1 D26309 May be a Limk1 seems to be Mizuno et al. LIMK (LIM component of an implicated in Oncogene kinase) intracellular williams syndrome 9: 1605-1612 signaling pathway (ws), a disease (1994). and may be characterized by involved in brain impaired visuospatial Development. constructive Displays serine/ cognition. threonine-specific phosphorylation of myelin basic protein and Histone (mbp) in vitro. 52 30948 22056 mRNA for D26309 LIMK (LIM kinase) 53 30896 22014 mRNA for PROTEIN- D26181 Can phosphorylate Palmer et al. novel protein KINASE ribosomal protein Eur J Biochem. kinase PKN C-RELATED s6. Mediates gtpase Jan. 15, 1995; KINASE 1; rho dependent 227(1-2): PROTEIN intracellular 344-51. KINASE signalling (by C-LIKE PKN; similarity). SERINE- THREONINE PROTEIN KINASE N 54 30900 22018 mRNA for PROTEIN- D26181 Can phosphorylate Palmer et al. novel protein KINASE ribosomal protein Eur J Biochem. kinase PKN C-RELATED s6. Mediates gtpase Jan. 15, 1995; KINASE 1; rho dependent 227(1-2): PROTEIN intracellular 344-51 KINASE signalling (by C-LIKE PKN; similarity). SERINE- THREONINE PROTEIN KINASE N 55 29052 20685 cell cycle related CDCH; AF035013 cholinergic signals in Jiang Y., Zhao K.; kinase CYCLIN- the hematopoietic Submitted DEPENDENT pathway--antisense (NOV-1997) to the PROTEIN oligonucleotide EMBL/GenBank/ KINASE H inhibited DDBJ databases. megakaryocyte development in bone marrow cultures. 56 29494 20894 mRNA for ZIPK AB007144 Serine/threonine Kawai et al. ZIP-kinase kinase which Mol Cell Biol. 1998 mediates apoptosis. Mar; 18(3): 1642-51. 57 29158 20739 diacylglycerol DGKI AF061936 Diacylglycerol Bowne et al. kinase iota (DAG) plays a (DGKi) central role in both the synthesis of **complex** lipids and in

intracellular signaling; may have important cellular functions in the retina and brain. 58 28184494 RYK = related to S59184 Potential growth Protooncogene for Wang et al. 23 receptor tyrosine factor receptor chicken sarcoma Mol Med. 1996 kinase protein tyrosine (new variant- Mar; 2(2): 189-203. [hepatoma] kinase. The soluble) expression of this receptor tyrosine kinase in Epithelial ovarian cancer suggests that it may be involved in tumor progression, which needs further investigation. 59 28191494 20001 RYK = related to S59184 Potential growth Protooncogene for Wang et al. 30 receptor tyrosine factor receptor chicken sarcoma Mol Med. 1996 kinase protein tyrosine (new variant- Mar; 2(2): 189-203. [hepatoma] kinase. The soluble) expression of this receptor tyrosine kinase in Epithelial ovarian cancer suggests that it may be involved in tumor progression, which needs further investigation. 60 31060 myotonin protein MYOTONIC S72883 May play a role in Defects in dmpk are Fu et al. kinase = DISTROPHY the intracellular the cause of myotonic Science thymopoietin PROTEIN communication. distrophy (dm), an 255: 1256-1258 homolog KINASE; autosomal dominant (1992). [muscle] MDPK; neurodegenerative DM-KINASE; disorder DMK; characterized by DMPK; myotonia, muscle MT-PK wasting in the distal extremities, cataract, hypogonadism, defective endocrine functions, male baldness, and cardiac arrhythmias. DM patients show decreased levels of kinase expression inversely related to repeat length. The minimum estimated incidence is 1 in 8000. 61 31084 myotonin protein MYOTONIC S72883 May play a role in Defects in dmpk are Fu et al. kinase = DISTROPHY the intracellular the cause of myotonic Science

PGPUB-DOCUMENT-NUMBER: 20020107252

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020107252 A1

TITLE: Novel Compounds

PUBLICATION-DATE: August 8, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Baxter, Andrew	Leicestershire		GB	
Brough, Stephen	Selston		GB	
Faull, Alan	Cheshire		GB	
Johnstone, Craig	Cheshire		GB	
McInally, Thomas	Leics		GB	

APPL-NO: 09/ 868884

DATE FILED: February 5, 2002

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
GB	0003154.2	2000GB-0003154.2	February 12, 2000

PCT-DATA:
APPL-NO: PCT/SE01/00248
DATE-FILED: Feb 7, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 514/252.01, 514/252.05 , 514/255.05 , 514/256 , 514/340 , 514/342 , 514/343 , 514/370 , 514/377 , 514/397 , 514/398 , 514/422 , 514/426 , 514/447 , 514/471 , 544/238 , 544/403 , 544/405 , 548/190 , 548/233 , 548/326.5 , 548/557

ABSTRACT:

The invention relates to heteroaromatic carboxamides of formula (I), 1 wherein A, R.sup.1, R.sup.2 and X are as defined in the specification, processes and intermediates used in their preparation, pharmaceutical compositions containing them and their use in therapy.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (164):

[0161] The compounds of formula (I) have activity as pharmaceuticals, in particular as **IKK2** enzyme inhibitors, and may be used in the treatment (therapeutic or prophylactic) of conditions/diseases in human and non-human animals in which inhibition of **IKK2** is beneficial. Examples of such conditions/diseases include inflammatory diseases or diseases with an inflammatory component. Particular diseases include inflammatory arthritides including rheumatoid arthritis, osteoarthritis, spondylitis, Reiters syndrome. psoriatic arthritis, lupus and bone resorptive disease: multiple sclerosis. inflammatory bowel disease including Crohn's disease; asthma, chronic obstructive pulmonary disease, emphysema, rhinitis, myasthenia gravis, Graves' disease, allograft rejection, psoriasis, dermatitis, allergic disorders, immune **complex** diseases, cachexia, ARDS, toxic shock, cardiovascular disorders, heart failure, myocardial infarcts, atherosclerosis, reperfusion injury, AIDS and cancer.

PGPUB-DOCUMENT-NUMBER: 20020086980

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020086980 A1

TITLE: Novel molecules of the card-related protein family and uses thereof

PUBLICATION-DATE: July 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bertin, John	Watertown	MA	US	

APPL-NO: 09/ 728260

DATE FILED: December 1, 2000

RELATED-US-APPL-DATA:

child 09728260 A1 20001201

parent continuation-in-part-of 09685791 20001010 US PENDING

child 09685791 20001010 US

parent continuation-in-part-of 09513904 20000225 US PENDING

child 09513904 20000225 US

parent continuation-in-part-of 09507533 20000218 US PENDING

non-provisional-of-provisional 60168780 19991201 US

US-CL-CURRENT: 536/23.1

ABSTRACT:

Novel CARD-9, CARD-10, or CARD-11 polypeptides, proteins, and nucleic acid molecules are disclosed. In addition to isolated CARD-9, CARD-10, or CARD-11 proteins, the invention further provides CARD-9, CARD-10, or CARD-11, fusion proteins, antigenic peptides and anti-CARD-9, CARD-10, or CARD-11 antibodies. The invention also provides CARD-9, CARD-10, or CARD-11 nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced and non-human transgenic animals in which a CARD-9, CARD-10, or CARD-11 gene has been introduced or disrupted. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. application Ser. No. 09/685,791, filed Oct. 10, 2000, which is a continuation-in-part of U.S. application Ser. No. 09/513,904, filed Feb. 25, 2000, which is a continuation-in-part of application Ser. No. 09/507,533, filed Feb. 18, 2000, which claimed priority from provisional application serial No. 60/168,780, filed Dec. 3, 1999. The content of each of these applications is herein incorporated by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (9):

[0009] Nuclear factor-.kappa.B (NF-.kappa.B) is a transcription factor that is expressed in many cell types and activates genes that have NF-.kappa.B sites in their promoters. Molecules that regulate NF-.kappa.B activation play a critical role in both apoptosis and in the stress-response of cells. With respect to stress-reponse, NF-.kappa.B activates genes that control immune defense mechanisms and inflammation. The CARD-containing proteins RICK, CARD-4 and Bcl-10 also induce activation of the NF-.kappa.B transcription factor suggesting that CARD/CARD signaling complexes regulate activation of the **IKK complex** (Inohara et al. 1998 Proc. Natl. Acad Sci. USA 273:12296; Bertin et al. 1999 J. Biol. Chem. 224:12955; Willis et al. 1999 Cell 96: 33). In unstimulated cells, NF-.kappa.B is found sequestered in the cytoplasm through interactions with inhibitory I.kappa.B proteins. Inhibition is relieved by the phosphorylation and proteosomal degradation of I.kappa.B proteins by proinflammatory cytokines. Phosphorylation is mediated by the **IKK complex** which is comprised of at least three major proteins: two kinases designated IKK.alpha. and IKK.beta. that directly phosphorylate the I.kappa.B inhibitory proteins, and a noncatalytic subunit called IKK.gamma. that functions to link the **IKKs** to upstream regulatory molecules (Zhang et al., 2000). Recently, RICK has been found to function as upstream regulatory molecules of the **IKK complex** (Inohara et al. 2000 J. Biol. Chem. 275:27823). RICK interacts directly with IKK.gamma. suggesting that it functions as signaling adaptor between the **IKK complex** and an upstream CARD-containing NF-.kappa.B activator. Indeed, CARD-4 forms a CARD/CARD signaling **complex** with RICK that induces activation of the **IKK complex** and the subsequent release of NF-.kappa.B (Bertin et al. 1999 J. Biol. Chem. 224:12955; Inohara et al. 1999 J. Biol. Chem. 274:14566; Inohara et al. 2000 J. Biol. Chem. 275:27823).

Detail Description Paragraph - DETX (72):

[0168] These results, taken with the finding of a direct interaction between CARD-9 and Bcl-10 suggest that CARD-9 is a specific regulator of Bcl-10 function. CARD-9 could play a role as an upstream signaling molecule that recruits Bcl-10 through CARD/CARD interactions. The resulting signaling **complex** may interact directly or indirectly with components of the **IKK complex** resulting in its activation, e.g., through oligomerization of IKK.gamma.. Indeed the data described above data shows that both CARD-9 and Bcl-10 form

large oligomeric complexes (filaments) when overexpressed in mammalian cells. Furthermore, enforced oligomerization of the C-terminus of Bcl-10/CLAP is thought to induce NF-.kappa.B activation, suggesting that the CARD domain of Bcl-10 functions as an oligomerization domain that transduces the activation signal to the **IKK complex** through its C-terminal domain. The ability of CARD-9 to form a **complex** with Bcl-10 via CARD/CARD interactions supports the idea that Bcl-10 functions as an adaptor between the effector **IKK complex** and the proximal signaling complexes that interact with CARD-9. Signaling molecules upstream of CARD-9 are predicted to transduce their signals to Bcl-10 through direct interactions with the C-terminal coiled-coil domain of CARD-9. Taken together, these results identify CARD-9 as an important mediator of NF-.kappa.B signaling through Bcl-10.

Detail Description Paragraph - DETX (77):

[0173] These studies showed that when CARD-11 is expressed in 293T cells, NF-kB activity is induced 20- to 40-fold compared to empty vector (FIG. 20A). NF-kB signaling occurred through the **IKK complex** since dominant-negative versions of **IKK-g and IKK-b** blocked the ability of CARD-11 to induce NF-kB activity (data not shown). To determine the role of individual domains in NF-.kappa.B signaling, a series of N- and C-terminal truncation mutants of CARD-11 were constructed (FIG. 20B). The N-terminal CARD of CARD-11 was essential for NF-.kappa.B signaling since deletion of this domain eliminated the induction of NF-kB activity (FIG. 20C). Immunoblot analysis revealed that the mutant proteins were expressed at levels similar to wt protein indicating that loss of function was not due to reduced levels of expression. In contrast, the C-terminal PDZ, SH3 and GUK domains were not required for NF-kB signaling since deletion of these domains had no effect on the ability of CARD-11 to induce NF-kB activity. However, a CARD-11 mutant lacking its C-terminal PDZ, SH3 and GUK domains induced NF-kB activity to levels 4- to 5-fold greater than that obtained with wt protein (FIG. 20C). Thus, the C-terminal domains may function to negatively regulate induction of NF-kB signaling by CARD-11.

Detail Description Paragraph - DETX (86):

[0182] CARD-11 is a specific regulator of Bcl-10 function. The finding that CARD-11 binds to Bcl-10 through a CARD/CARD interaction suggests that this molecule functions as upstream activator of Bcl-10. As discussed above, CARD-9 also binds to the CARD activation domain of Bcl-10 and signals NF-kB activation. Thus, CARD11and CARD-9 constitute a subclass of CARD proteins that may function to transduce upstream stimuli to the activation of Bcl-10 and NF-kB. In response to upstream signals, the coiled-coil domains could mediate self-association of CARD-11 resulting in the aggregation and activation of Bcl-10. Bcl-10 might then engage and oligomerize **IKKg** resulting in the activation of the **IKK complex** and NF-kB (Inohara et al. 1999 J. Biol. Chem. 274:14566; Poyet et al., 1999). Thus, CARD-11 could function in a manner analogous to Apaf-1 and CARD-4 that function as upstream regulators to induce oligomerization and activation of their respective downstream CARD binding partners. The data showing that CARD-11 induces the phosphorylation of Bcl-10 suggests that signal transduction may involve the participation of a

serine/threonine kinase. The C-terminal PDZ/SH3/GUK domains of CARD-11 may function in an analogous manner to the C-terminal LRR domain of CARD-4 and the WD-40 domain of Apaf-1 to regulate protein activation by upstream signals. PDZ/SH3/GUK domains identify MAGUK family members, a class of proteins that associate with the plasma membrane (Fanning and Anderson, 1999 Curr Opin Cell Biol 11:432-9). Interestingly, the PDZ domain found in many MAGUK proteins has been shown to interact with the intracellular domains of specific receptors. Thus, CARD-11 may function as a scaffolding protein to assemble a multi-protein **complex** at the intracellular domain of a receptor that signals the activation of NF-kB.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020081636 A1

TITLE: Novel molecules of the card-related protein family and uses thereof

PUBLICATION-DATE: June 27, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bertin, John	Watertown	MA	US	

APPL-NO: 09/ 767215

DATE FILED: January 22, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60181159 20000209 US

US-CL-CURRENT: 435/7.23, 435/183 , 530/388.26

ABSTRACT:

Novel CARD-14 polypeptides, proteins, and nucleic acid molecules are disclosed. In addition to isolated CARD-14 proteins, the invention further provides CARD-14 fusion proteins, antigenic peptides and anti-CARD-14 antibodies. The invention also provides CARD-14 nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced and non-human transgenic animals in which a CARD-14 gene has been introduced or disrupted. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.

RELATED APPLICATION INFORMATION

[0001] This application claims priority from provisional application Ser. No. 60/181,159, filed Feb. 9, 2000, the entire content of which is incorporated herein by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (37):

[0099] CARD-14 is a specific regulator of Bcl-10 function. The finding that CARD-14 binds to Bcl-10 through a CARD/CARD interaction suggests that this

molecule functions as upstream activator of Bcl-10. Thus, CARD-14 is a member of a class of CARD proteins that may function to transduce upstream stimuli to the activation of Bcl-10 and NF-kB. In response to upstream signals, the coiled-coil domains could mediate self-association of CARD-14, resulting in the aggregation and activation of Bcl-10. Bcl-10 might then engage and oligomerize **IKKg** resulting in the activation of the **IKK complex** and NF-kB (Inohara et al. 1999 J. Biol. Chem. 274:14566; Poyet et al., 1999). Thus, CARD-14 could function in a manner analogous to Apaf-1 and CARD-4 that function as upstream regulators to induce oligomerization and activation of their respective downstream CARD binding partners. The data showing that CARD-14 induces the phosphorylation of Bcl-10 suggests that signal transduction may involve the participation of a serine/threonine kinase. The C-terminal PDZ/SH3/GUK domains of CARD-14 may function in an analogous manner to the C-terminal LRR domain of CARD-4 and the WD-40 domain of Apaf-1 to regulate protein activation by upstream signals. PDZ/SH3/GUK domains identify MAGUK family members, a class of proteins that associate with the plasma membrane (Fanning and Anderson, 1999 Curr Opin Cell Biol 11:432-9). Interestingly, the PDZ domain found in many MAGUK proteins has been shown to interact with the intracellular domains of specific receptors. Thus, CARD-14 may function as a scaffolding protein to assemble a multi-protein **complex** at the intracellular domain of a receptor that signals the activation of NF-kB.

Detail Description Paragraph - DETX (40):

[0102] The binding of CARD-14 to Bcl-10 described above suggests that CARD-14-Bcl-10 interactions may be part of a signaling pathway involved in apoptosis and NF-KB activation. Consistent with this signal transduction model, CARD-14 was shown to be an inducer of NF-KB activation. Expression of CARD-14 in 293T cells resulted in a 20-40 fold increase in NF-KB activity, compared to empty vector (FIG. 9A). NF-kB signaling occurred through the **IKK complex** since dominant-negative versions of **IKK-g and IKK-b** blocked the ability of CARD-14 to induce NF-kB activity.

PGPUB-DOCUMENT-NUMBER: 20020072523

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020072523 A1

TITLE: Amino-substituted tetracyclic compounds useful as anti-inflammatory agents and pharmaceutical compositions comprising same

PUBLICATION-DATE: June 13, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Beaulieu, Francis	Laprairie	CT	CA	
Ouellet, Carl	Boucherville	CT	CA	
Belema, Makonen	New Haven	CT	US	
Qiu, Yuping	Windsor	CT	US	
Yang, Xuejie	Middletown		US	
Zusi, Fred C.	Hamden		US	

APPL-NO: 09/ 965977

DATE FILED: September 27, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60223304 20001003 US

US-CL-CURRENT: 514/248, 514/257 , 544/247 , 544/343

ABSTRACT:

Compounds of formula (I), or pharmaceutically-acceptable salts thereof, are useful in treating inflammatory and immune diseases and disorders, 1 wherein X, Y.sub.1, Y.sub.2, and R.sub.2-R.sub.4 are as defined in the specification.

RELATED INVENTIONS

[0001] This application claims the benefit of U.S. application Serial No. 60/223,304, filed Oct. 3, 2000, pursuant to 35 U.S.C. .sctn. 119(e).

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (6):

[0005] Potential inhibitors of NF-.kappa.B and/or the NF-.kappa.B pathway have been identified as including Interleukin-10, glucocorticoids, salicylates,

nitric oxide, and other immunosuppressants. I.kappa.B is a cytoplasmic protein that controls NF-.kappa.B activity by retaining NF-.kappa.B in the cytoplasm. IKB is phosphorylated by the I.kappa.B kinase (**IKK**), which has two isoforms, **IKK**-.alpha. (or "**IKK-1") and IKK**-.beta. (or **IKK-2**). Upon phosphorylation of I.kappa.B by **IKK,** NF-.kappa.B is rapidly released into the cell and translocates to the nucleus where it binds to the promoters of many genes and up-regulates the transcription of pro-inflammatory genes. Glucocorticoids reportedly inhibit NF-.kappa.B activity by two mechanisms, i.e., upregulating I.kappa.B protein levels and inhibiting NF-.kappa.B subunits. Nitric oxide also reportedly inhibits NF-.kappa.B through upregulation of I.kappa.B. However, these mechanisms of interaction are **complex**; for example, production of nitric oxide in lymphocytes reportedly enhances NF-.kappa.B activity.

PGPUB-DOCUMENT-NUMBER: 20020068063

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020068063 A1

TITLE: Proteolysis targeting chimeric pharmaceutical

PUBLICATION-DATE: June 6, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Deshaies, Raymond J.	Claremont	CA	US	
Crews, Craig	New Haven	CT	US	
Sakamoto, Kathleen M.	Manhattan Beach	CA	US	

APPL-NO: 09/ 953473

DATE FILED: September 10, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60231359 20000908 US

US-CL-CURRENT: 424/178.1, 435/226

ABSTRACT:

The present invention is based on the discovery of a composition that provides targeted ubiquitination. Specifically the composition contains an ubiquitin pathway protein binding moiety which recognizes an ubiquitin pathway protein and a targeting moiety which recognizes a target protein. In addition, the present invention provides libraries of compositions, where each composition contains an ubiquitin pathway protein binding moiety and a member of a molecular library. The libraries of the present invention can be used to identify proteins involved in a predetermined function of cells.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. 119(e)(1) to U.S. Provisional Application No. 60/231,359, filed on Sep. 8, 2000, which is incorporated herein by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (6):

[0024] According to one embodiment of the present invention, an ubiquitin pathway protein binding moiety of the present invention is any suitable ligand

to an ubiquitin pathway protein, e.g., ubiquitin protein ligase or E3 protein or homologues thereof. In another embodiment, an ubiquitin pathway protein binding moiety of the present invention is any ubiquitin pathway protein binding peptide, domain or region of an ligand to an ubiquitin pathway protein. In still another embodiment, an ubiquitin pathway protein binding moiety of the present invention recognizes and binds to an ubiquitin pathway protein in a regulated manner. For example, IkBa is a well defined ligand for mammalian SCF.sup..beta.-TRCP **complex**. The mammalian F box protein, .beta.-TRCP/E3RS, has been shown to bind IkBa, a negative regulator of NFkB (See Yaron, A., et al., Nature (London), 396:590-94 (1998)). The SCF.sup..beta.-TRCP **complex** promotes the ubiquitination and subsequent degradation of IkBa, which results in activation of NFkB during the inflammatory response (See Deshaies, R. J., Annu. Rev. Cell Dev. Biol., 15:435-67 (1999)). The recruitment of IkBa to SCF.sup..beta.-TRCP is mediated by a ubiquitin pathway protein binding domain, which is a 10-aa peptide within IkBa, DRHDSGLDSM (See Yaron, A., et al., Nature (London), 396:590-94 (1998); and Yaron, A., et al., EMBO J., 16: 6486-94 (1997)). In response to diverse inflammatory signals, IkBa kinase (**IKK**) phosphorylates this motif on both serines, which triggers the binding of IkBa to .beta.-TRCP.

Detail Description Paragraph - DETX (41):

[0051] Before testing the activity of Protac-1, an approach to isolate and assay SCF.sup..beta.-TRCP complexes in vitro as described (See Tan, P., et al., Mol. Cell, 3:327-33 (1999)) was first adapted. Lysates from 293T cells transfected with plasmids that encoded .beta.-TRCP and Cul-1 proteins tagged with the FLAG epitope at the N terminus were immunoprecipitated with anti-FLAG antibody-conjugated resin. Immunoblot analysis confirmed that all components of SCF.sup..beta.-TRCP were present in the anti-FLAG immunoprecipitate, including Skpl, Hrtl, and the transfected FLAG Cul-1 and FLAG .beta.-TRCP (data not shown). Furthermore, control experiments confirmed that these immunoprecipitates promoted ubiquitination of **IKK**-phosphorylated glutathione S-transferase-IkBa in a manner that was inhibited by the IPP and Protac-1 (data not shown; ref 7). To determine whether Protac-1 could recruit MetAP-2 to SCF.sup..beta.-TRCP, MetAP-2 (18 .mu.M) was first incubated with Protac-1 (100 .mu.M) for 45 min at room temperature. After isolation of SCF.sup..beta.-TRCP complexes, the anti-FLAG beads were supplemented with the MetAP-2--Protac-1 mixture and rotated at room temperature for 1 hr. The beads and supernatant then were evaluated by Western blot analysis for the presence of MetAP-2. Anti-FLAG beads coated with SCF.sup..beta.-TRCP, but not control beads preincubated with untransfected 293T cell lysates, specifically retained a fraction of the MetAP-2--Protac-1 **complex** and not the unliganded MetAP-2 (FIG. 3). These results demonstrate that Protac-1 specifically recruited MetAP-2 to SCF.sup..beta.-TRCP.

Detail Description Paragraph - DETX (45):

[0053] The experiments described above demonstrated that MetAP-2 was ubiquitinated in a Protac-1-dependent manner by highly purified SCF.sup..beta.-TRCP. However, the key issues are whether Protac-1 can specifically activate MetAP-2 degradation, and whether targeted degradation can

be achieved by endogenous ubiquitin/proteasome pathway components at typical intracellular concentrations. To address these questions, MetAP-2 was preincubated with Protac-1 to allow the complexes to form, and then added the mixture to Xenopus egg extract supplemented with **IKK**-EE (See Mercurio, F., Zhu, et al., Science, 278:860-66. (1997)), OA, and OVA. The addition of **IKK**-EE and OA was intended to sustain phosphorylation of the IkBa peptide moiety of Protac-1, whereas OVA was added to prevent the further linkage of Protac-1 to MetAP-2. Remarkably, MetAP-2--Protac-1 **complex** (top band) but not MetAP-2 alone (bottom band) was mostly degraded after 30 min (FIG. 5). Degradation of MetAP-2--Protac-1 was attenuated in extracts supplemented with the proteasome inhibitors LLnL or epoxomicin (See Meng, L., et al., Proc. Natl. Acad. Sci. USA, 96:10403-08 (1999)), but not by other protease inhibitors (chymotrypsin, pepstatin, and leu-peptin) added to the reaction. Moreover, addition of both **IKK**-EE and OA was required for optimal degradation of MetAP-2--Protac-1. Similarly, we have seen specific turnover of the MetAP-2--Protac-1 **complex,** but not free MetAP-2, in three independent experiments. Because the IPP does not have lysines and the OVA does not have free amino groups, it is unlikely that Protac itself serves as a target for ubiquitin-dependent proteolysis. Taken together, these results demonstrate that Protac-1 targeted MetAP-2 for degradation by means of the proteasome. MetAP-2 turnover was very specific, in that maximal degradation required agents predicted to sustain phosphorylation of the IkBa peptide.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020058077 A1

TITLE: Cancer chemotherapeutical and chemopreventive agent

PUBLICATION-DATE: May 16, 2002

INVENTOR-INFORMATION:

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APPL-NO: 10/ 003746

DATE FILED: November 14, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60248962 20001115 US

US-CL-CURRENT: 424/764

ABSTRACT:

This invention relates to the use of parthenolide or derivative thereof and chrysanthemum ethanolic extract containing parthenolide in the treatment and prevention of cancer, including cancer associated with an increased COX-2 expression and increased constitutive activation of NF-.kappa.B.

RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 60/248,962, filed Nov. 15, 2000.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (5):

[0004] Parthenolide, as shown in FIG. 1, contains an .alpha.-methylene-.gamma.-lactone ring and an epoxide which are able to interact readily with nucleophilic sites of biological molecules. Parthenolide possesses remarkable anti-inflammatory property and one of the important mechanisms is related to its inhibitory effect on arachidonic acid metabolism and prostaglandin (PG) production through its direct interaction with cyclooxygenase (COX) enzyme (Capasso, 1986; Pugh and Sambo, 1988; Sumner et al., 1992) or suppression of COX-2 expression via the inhibition of protein

tyrosine kinase in lipopolysaccharide (LPS)-stimulated macrophages (Hwang et al., 1996). Parthenolide has also been shown to be a potent nuclear factor-.kappa.B (NF-.kappa.B) inhibitor as it can specifically suppress the activity of the **IKK complex** and the subsequent degradation of the NF-.kappa.B inhibitory proteins (I.kappa.B.alpha. and I.kappa.B.beta.) (Bork et al., 1997; Hehner et al., 1998; 1999). COX-2 is one of the target genes regulated by NF-.kappa.B (Appleby et al., 1994; Tazawa et al., 1994; Yamamoto et al., 1995), but it is not known whether parthenolide is capable of inhibiting COX expression and PG production by its effect on NF-.kappa.B or whether parthenolide is capable of inhibiting COX expression in cancer cells.

Detail Description Paragraph - DETX (67):

[0089] In this part of the experiment, we further studied effects of parthenolide on the sequential events including (i) cytoplasmic I.kappa.B.alpha. degradation, (ii) p65 nuclear translocation and (iii) NF-.kappa.B-DNA binding in TNF-.alpha. treated CNE1 cells. When cells were treated with TNF-.alpha. for 30 min, the unphosphorylated I.kappa.B.alpha. in the cytosolic fraction was completely degraded, accompanied by the significant increase of the amount of p65 and DNA binding activity in the nuclear, detected by western blot and EMSA, respectively (FIGS. 6A and 6B, lane 2). Parthenolide pre-treatment alone (25 .mu.M.times.4 h) does not cause any of these changes (FIGS. 6A and 6B, lane 3). In order to optimise the parthenolide pre-treatment condition, CNE1 cells were first pretreated with parthenolide (25 .mu.M) for a period ranging from 0 to 4 h. As shown in FIG. 6A, no protective effects were found when parthenolide was added with TNF-.alpha. simultaneously (lane 4) or with 0.5 h pre-treatment (lane 5). The protective effect was seen from 1 h onwards and 4 h pre-treatment offers the most significant inhibitory effect against I.kappa.B.alpha. degradation, p65 nuclear translocation and DNA binding (lane 8). Therefore, cells were pretreated with parthenolide for 4 h prior to TNF-.alpha. exposure in the subsequent dose-response study with results summarized in FIG. 6B. No evident inhibitory effects were found in the two lower doses (5 and 10 .mu.M) (FIG. 6B, lanes 4 and 5), while higher concentrations of parthenolide (from 15 to 25 .mu.M) significantly suppressed TNF-.alpha. induced I.kappa.B.alpha. degradation, NF-.kappa.B nuclear translocation and DNA binding in a dose-dependent manner (FIG. 6B, lanes 6, 7 and 8). Parthenolide has been found to act on the upstream kinases of I.kappa.B (**IKK complex**) to inhibit NF-.kappa.B activation (Hehner et al., 1999). Although the direct effect of parthenolide on **IKK** is not examined in the present study, the dose-dependent inhibition of parthenolide on I.kappa.B.alpha. degradation suggests that parthenolide may also act through a similar pattern to suppress the phosphorylation, ubiquitination and degradation of this inhibitor, which eventually prevents NF-.kappa.B activation in CNE1 cells.

Detail Description Paragraph - DETX (131):

[0150] Israel, A. (2000) The **IKK complex**: an integrator of all signals that activate NF-kappaB? Trends Cell Biol. 10, 129-133

Detail Description Paragraph - DETX (149):

[0168] Plummer, S. M., Holloway, K. A., Manson, M. M., Munks, R. J., Kaptein, A., Farrow, S., and Howells, L. (1999) Inhibition of cyclo-oxygenase 2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF-kappaB activation via the NIK/**IKK** signalling **complex**. Oncogene 18, 6013-6020

PGPUB-DOCUMENT-NUMBER: 20020056150

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020056150 A1

TITLE: Mutations in IKK gamma

PUBLICATION-DATE: May 9, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Karin, Michael	La Jolla	CA	US	

APPL-NO: 09/ 882507

DATE FILED: June 15, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60212438 20000616 US

US-CL-CURRENT: 800/18, 800/9

ABSTRACT:

The present invention relates to compositions and methods involving IKK.gamma. mutants. In particular, the present invention provides methods and compositions, including transgenic animals, suitable for use in determining means to treat, control, and/or prevent incontinentia pigmenti (IP). The present invention also provides methods to detect the presence of mutations in the IKK.gamma. gene and protein.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit under 35 U.S.C. .sctn.119(e) of provisional patent U.S. Ser. No. 60/212,438, filed on Jun. 16, 2000, which is herein incorporated by reference in its entirety for all purposes.

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Brief Description of Drawings Paragraph - DRTX (2):

[0012] FIG. 1 shows various aspects involved in the generation of IKK.gamma./NEMO-deficient mice. In Panel A, maps of the mouse Ikk.gamma./NEMO locus, targeting vectors, and the targeted allele generated by homologous integration of vector 1 are provided. In this Figure, exons 1 and 2 are indicated by the solid boxes. The translation start site, selection markers,

hybridization probe (bold line), PCR screening primers (P1 and P2), and restriction enzyme sites are shown (B, BamHI; Bg, Bg/II; 47III, Eco47III; S, SalI). Panel B provides the results of Western blot analysis of **IKK** subunits in whole embryonic stem (ES) cell extracts. In this Figure, lane 1 contains wild-type, lane 2 contains Ikk.gamma..sup.- from KO1, lane 3 contains Ikk.gamma..sup.- ES2 from KO1, lane 4 contains Ikk.gamma..sup.- ES3 from KO2, and lane 5 contains Ikk.gamma..sup.- ES4 from KO2; IKK.gamma.(N) and IKK.gamma.(C) refer to antibodies directed against the N- and C-terminal regions of IKK.gamma./NEMO, respectively. Panel C provides results of experiments in which murine embryonic fibroblasts (MEFs) derived from wild-type and Ikk.gamma..sup.- E12 embryos were treated with 10 ng/ml tumor necrosis factors (TNF.alpha.) or 20 ng/ml interleukin-1 (IL-1). At the indicated times, cells were lysed and **IKK** activity (KA) was measured by immune **complex** kinase assay using IKK.alpha. antibodies and glutathione-s-transferase fusion protein, GST-1.kappa.B.alpha. (1-54), as a substrate. The reaction products were separated by a 10% SDS-PAGE, transferred to a membrane, and autoradiographed. The membrane was reprobed (Blot: IKK.alpha.) with a monoclonal antibody against IKK.alpha. as a loading control. Panel D shows the NF-.kappa.B binding activity in 20 .mu.g of whole cell extracts prepared as described in Example 3. NF-1 probe binding was used to control the quality and quantity of the nuclear protein extract. Panel E illustrates TNF.alpha.-induced cytotoxicity for wild-type and Ikk.gamma. MEFs treated with mouse TNF.alpha. (50 ng/ml). At the indicated time points, cells were fixed, stained with DAPI, and mounted with a coverslip. Values shown are percentages of apoptotic nuclei scored using a fluorescent microscope.

Detail Description Paragraph - DETX (28):

[0050] The **IKK Complex**

Detail Description Paragraph - DETX (29):

[0051] The **IKK complex** is composed of the IKK.alpha. and IKK.beta. catalytic subunits (DiDonato et al., Nature 388:548-554 [1997]; Mercurio et al., Science 278:860-866 [1997]; Regnier et al., Cell 90:373-383 [1997]; and Zandi et al., Cell 91:243-252 [1997]), and the IKK.gamma./NEMO regulatory subunit (Rothwarf et al., Nature 395:297-300 [1998]; and Yamaoko et al., Cell 93:1231-1240 [1998]). This **complex** is essential for activation of NF-.kappa.B transcription factors (Rothwarf and Karin, Science's STKE [1999]). NF-.kappa.B activation by proinflammatory stimuli is required for induction of genes whose products are involved in both innate and adaptive immunity (Ghosh et al., Ann. Rev. Immunol. 16:225-260 [1998]). NF-.kappa.B target genes include chemokines, cytokines, adhesion molecules and enzymes that produce secondary inflammatory mediators. In addition, NF-.kappa.B activation is required for protection of cells from apoptosis, especially that caused by members of the tumor necrosis factor (TNF) family of death cytokines (Beg and Baltimore, Science 274:782-784 [1996]; Liu et al., Cell 87:565-576 [1996]; Van Antwerp et al., Science 274:787-789 [1996]; and Wang et al., Science 274:784-787 [1996]). Purified recombinant IKK.alpha. and IKK.beta. both phosphorylate the I.kappa.B inhibitors of NF-.kappa.B, at sites that cause their ubiquitin-mediated degradation in vivo (Zandi et al., Science 281:1360-1363

[1998]). However, IKK.alpha.:IKK.beta. dimers that form in the absence of IKK.gamma./NEMO, and even those that associate with C-terminally truncated IKK.gamma./NEMO, are refractory to most NF-.kappa.B activators, including interleukin 1 (IL-1), double-stranded (ds) RNA, bacterial lipopolysaccharide (LPS), and TNF.alpha. (Rothwarf et al., supra [1998]; and Yamaoka et al., supra [1998]). As a result, IKK.gamma./NEMO-deficient cells are practically devoid of NF-.kappa.B activity, even after cell stimulation (Yamaoka et al, supra [1998]).

Detail Description Paragraph - DETX (30):

[0052] Genetic analysis of **IKK** function through gene targeting in mice revealed that despite the high degree of sequence similarity and identical substrate specificity, the two catalytic subunits of the **IKK complex** dramatically differ in their biological functions. IKK.alpha. is required for proper development and differentiation of the epidermis and other ectodermal derivatives, but is not required for **IKK** activation by proinflammatory stimuli (Hu et al., Science 284:316-320 [1999]; and Takeda et al., Science 284:313-316 [1999]), nor for induction of NF-.kappa.B activity in the affected cell types. Therefore, it is unlikely that IKK.alpha. function in control of keratinocyte differentiation is exerted via NF-.kappa.B. In contrast, IKK.beta. is essential for **IKK** and NF-.kappa.B activation by proinflammatory stimuli. Ikkb.sup.-/- mice die at mid-gestation due to massive liver apoptosis and IKK.beta.-deficient cells are sensitive to TNF.alpha.-induced apoptosis (Li et al., Proc. Natl. Acad. Sci. USA 96:1042-1047 [1999], Li et al., Genes Dev. 13:1322-1328 [1999]; and Tanaka et al. Immunity 10:421-429 [1999]). This phenotype is essentially identical to that of RelA.sup.-/- mice which lack the p65 subunit of NF-.kappa.B (Beg et al., Nature 376:167-169 [1995]). However, an understanding of the mechanism(s) is not necessary in order to make and use the present invention.

Detail Description Paragraph - DETX (32):

[0054] Unlike the genes coding for the catalytic subunits of the **IKK complex,** the IKK.gamma./NEMO gene, which codes for the essential IKK.gamma./NEMO regulatory subunit is located on the X-chromosome. Due to this chromosomal location and random X chromosome inactivation (Lyonization), loss-of-function Ikk.gamma./NEMO mutations result in different phenotypes in female and male mice. While mutant males die in utero, as expected for a complete **IKK** and NF-.kappa.B deficiency (Li et al., Proc. Natl. Acad. Sci. USA 96:1042-1047 [1999], Li et al., Genes Dev. 13:1322-1328 [1999]; and Tanaka et al. Immunity 10:421-429 [1999]), heterozygous mutant females are born alive, but display a severe, yet transient, multi-organ disease. The cutaneous signs of the disease detected in Ikk.gamma..sup.+/- female mice are very similar to those observed in human patients suffering from IP. Both Ikk.gamma..sup.+/- mice and female IP patients eventually recover, reach sexual maturity, and can transmit the disease to the next generation. Like Ikk.gamma./NEMO mutant mice and cells, cells and tissues from IP patients exhibit defective IKK.gamma./NEMO expression.

Detail Description Paragraph - DETX (43):

[0065] Like most other NF-.kappa.B-deficient cells, the IKK.gamma./NEMO-deficient keratinocytes are very sensitive to apoptosis; TUNEL assays revealed a large increase in the frequency of apoptotic cells in the epidermis of Ikk.gamma..sup.+/- mice. Increased apoptosis was not limited to the epidermis, but was also seen in the thymus and spleen. In all of these cases, apoptosis may be enhanced or driven by death cytokines produced by neighboring IKK.gamma./NEMO-positive cells. Many of the TUNEL-positive cells in the epidermis of Ikk.gamma..sup.+/- mice were clustered and appeared to be extruded from the epidermis by growth of the remaining normal cells. Most likely, this process contributes to the shedding of dead skin fragments and the eventual disappearance of the verrucous lesions. The third stage of the disease is characterized by streaks of hyperpigmentation and scarring (Landy and Donnai, J. Med. Genet. 30:53-59 [1993]; and Francis and Sybert, Semin. Cutan. Med. Surg. 16:54-60 [1997]). Histological examination reveals loss (incontinence) of melanin from basal cells of the epidermis and the appearance of free pigment deposits, or melanin-containing macrophages (Francis and Sybert, supra [1997]; and Scheuerle, J. Med. Genet. 77:201-218 [1998]). Although an understanding of the mechanism(s) is not necessary in order to use the present invention, the loss of pigment cells appears to be due to the apoptosis and necrosis of IKK.gamma./NEMO-deficient cells that release free melanin, some of which is ingested by macrophages. Eventually, all or most of the IKK.gamma./NEMO-deficient cells are eliminated through apoptosis and wherever possible, are replaced by surviving and proliferation-competent IKK.gamma./NEMO-expressing cells (See, FIG. 8). However, it is believed that some residual IKK.gamma./NEMO-deficient cells are capable of giving rise to another disease cycle, as has been occasionally observed (van Leeuwen et al., Pediatr. Dermatol. 17:70 [2000]). The importance of the **IKK complex** and NF-.kappa.B for prevention of apoptosis in humans, as well as mice is underscored by the contemplated association between human IP and IKK.gamma./NEMO-deficiency.

Detail Description Paragraph - DETX (67):

[0083] In these experiments, MEFs and ES cell extracts were examined. Wild-type and Ikk.gamma. mutant MEFs and ES cells were treated with TNF.alpha. (10 ng/ml) or IL-1 (20 ng/ml). Control cells (i.e., without TNF.alpha. or IL-1 treatment) were also included. At the times indicated in FIG. 1, Panel C, the **IKK complex** was immunoprecipitated from cell lysates with a polyclonal antibody directed against IKK.alpha. (M280, Santa Cruz), and its activity measured by an immunocomplex kinase assay with GST-1.kappa.B.alpha. (1-54) as a substrate, as known in the art (See, Rothwarf et al., Nature 395:297-300 [1998]). Loading was normalized by immunoblotting with a monoclonal antibody against IKK.alpha. (1:500; Imgenex). Immunoblot analysis was conducted as known in the art and previously described (See, Hu et al., Science 284:316-320 [1999]). Electrophoretic mobility shift assays (EMSA) were performed as known in the art (See, Rothwarf et al., supra [1998]).

PGPUB-DOCUMENT-NUMBER: 20020049300

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020049300 A1

TITLE: IRAK3 polypeptides, polynucleotides and methods

PUBLICATION-DATE: April 25, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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APPL-NO: 09/ 863549

DATE FILED: May 22, 2001

RELATED-US-APPL-DATA:

child 09863549 A1 20010522

parent division-of 09135232 19980817 US GRANTED

parent-patent 6262228 US

US-CL-CURRENT: 530/300, 435/325 , 435/69.1 , 435/7.92 , 536/23.5

ABSTRACT:

The invention provides methods and compositions relating to a novel kinase, IRAK3. The polypeptides may be produced recombinantly from transformed host cells from the disclosed IRAK3 encoding nucleic acids or purified from human cells. The invention provides isolated IRAK3 hybridization probes and primers capable of specifically hybridizing with the disclosed IRAK3 genes, IRAK3-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (5):

[0004] Interleukin 1 (IL-1) receptor associated kinase (IRAK) functions as an intracellular signal transducer for the pro-inflammatory cytokine IL-1. IL-1 treatment of cells induces the **complex** formation of the two IL-1 receptor chains, IL-1R1 and IL-1RAcP, which recruits an adaptor molecule designated as MyD88 which binds to IRAK. IRAK is subsequently phosphorylated, released from the receptor **complex** to interact with TRAF6. TRAF6 triggers either the NIK/**IKK**

kinase cascade to activate the transcription factor NF-.kappa.B or an undefined kinase cascade to activate the transcription factor AP-1. Both transcription factors regulate large numbers of genes that regulate immune and inflammatory responses.

PGPUB-DOCUMENT-NUMBER: 20020045235

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020045235 A1

TITLE: IKB kinase, subunits thereof, and methods of using same

PUBLICATION-DATE: April 18, 2002

INVENTOR-INFORMATION:

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DiDonato, Joseph A.	Westlake	OH	US	
Rothwarf, David M.	La Jolla	CA	US	
Hayakawa, Makio	Tokyo	CA	JP	
Zandi, Ebrahim	Duarte		US	

APPL-NO: 09/ 796872

DATE FILED: February 28, 2001

RELATED-US-APPL-DATA:

child 09796872 A1 20010228

parent division-of 09168629 19981008 US GRANTED

parent-patent 6242253 US

non-provisional-of-provisional 60061470 19971009 US

US-CL-CURRENT: 435/194, 435/320.1 , 435/325 , 536/23.2

ABSTRACT:

The present invention provides an isolated nucleic acid molecules encoding I.kappa.B kinase (**IKK**) catalytic subunit polypeptides, which are associated with an **IKK** serine protein kinase that phosphorylates a protein (I.kappa.B) that inhibits the activity of the NF-.kappa.B transcription factor, vectors comprising such nucleic acid molecules and host cells containing such vectors. In addition, the invention provides nucleotide sequences that can bind to a nucleic acid molecule of the invention, such nucleotide sequences being useful as probes or as antisense molecules. The invention also provides isolated **IKK** catalytic subunits, which can phosphorylate an I.kappa.B protein, and peptide portions of such **IKK** subunit. In addition, the invention provides anti-**IKK** antibodies, which specifically bind to an **IKK complex or an IKK** catalytic subunit, and **IKK**-binding fragments of such antibodies. The invention further provides methods of substantially purifying an **IKK complex,** methods of identifying an agent that can alter the association of an **IKK complex or an IKK** catalytic subunit with a second protein, and methods of identifying proteins

that can interact with an **IKK complex or an IKK** catalytic subunit.

[0001] This application is based on, and claims the benefit of, U.S. Provisional Application No. 60/061,470, filed Oct. 9, 1997, the entire contents of which is herein incorporated by reference.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

The present invention provides an isolated nucleic acid molecules encoding I.kappa.B kinase (**IKK**) catalytic subunit polypeptides, which are associated with an **IKK** serine protein kinase that phosphorylates a protein (I.kappa.B) that inhibits the activity of the NF-.kappa.B transcription factor, vectors comprising such nucleic acid molecules and host cells containing such vectors. In addition, the invention provides nucleotide sequences that can bind to a nucleic acid molecule of the invention, such nucleotide sequences being useful as probes or as antisense molecules. The invention also provides isolated **IKK** catalytic subunits, which can phosphorylate an I.kappa.B protein, and peptide portions of such **IKK** subunit. In addition, the invention provides anti-**IKK** antibodies, which specifically bind to an **IKK complex or an IKK** catalytic subunit, and **IKK**-binding fragments of such antibodies. The invention further provides methods of substantially purifying an **IKK complex**, methods of identifying an agent that can alter the association of an **IKK complex or an IKK** catalytic subunit with a second protein, and methods of identifying proteins that can interact with an **IKK complex or an IKK** catalytic subunit.

Summary of Invention Paragraph - BSTX (15):

[0015] The present invention also provides isolated full length human **IKK** subunits, which can phosphorylate an I.kappa.B protein. For example, the invention provides an IKK.alpha. polypeptide having the amino acid sequence shown as SEQ ID NO: 2, particularly the amino acid sequence comprising amino acids 1 to 31 at the N-terminus of the polypeptide of SEQ ID NO: 2. In addition, the invention provides an IKK.beta. polypeptide having the amino acid sequence shown as SEQ ID NO: 15. The invention also provides peptide portions of an **IKK** subunit, including, for example, peptide portions comprising one or more contiguous amino acids of the N-terminal amino acids shown as residues 1 to 31 in SEQ ID NO: 2. A peptide portion of an **IKK** subunit can comprise the kinase domain of the **IKK** subunit or can comprise a peptide useful for eliciting production of an antibody that specifically binds to an I.kappa.B kinase or to the **IKK** subunit. Accordingly, the invention also provides anti-**IKK** antibodies that specifically bind to an **IKK complex** comprising an **IKK** subunit, particularly to the **IKK** subunit, for example, to an epitope comprising at least one of the amino acids shown as residues 1 to 31 of SEQ ID NO: 2, and also provides **IKK** subunit-binding fragments of such antibodies. In addition, the invention provides cell lines producing anti-**IKK** antibodies or **IKK**-binding fragments thereof.

Summary of Invention Paragraph - BSTX (16):

[0016] The invention also provides isolated I.kappa.B kinase complexes. As disclosed herein, an **IKK complex** can have an apparent molecular mass of about 900 kDa or about 300 kDa. An **IKK complex** is characterized, in part, in that it comprises an IKK.alpha. subunit, an IKK.beta. subunit, or both and can phosphorylate an I.kappa.B protein.

Summary of Invention Paragraph - BSTX (17):

[0017] The present invention further provides methods for isolating an **IKK complex or an IKK** subunit, as well as methods of identifying an agent that can alter the association of an **IKK complex or an IKK** subunit with a second protein that associates with the **IKK** in vitro or in vivo. Such a second protein can be, for example, another **IKK** subunit; an I.kappa.B protein, which is a substrate for **IKK** activity and is involved in a signal transduction pathway that results in the regulated expression of a gene; a protein that is upstream of the I.kappa.B kinase in a signal transduction pathway and regulates **IKK** activity; or a protein that acts as a regulatory subunit of the I.kappa.B kinase or of an **IKK** subunit and is necessary for full activation of the **IKK complex**. An agent that alters the association of an **IKK** subunit with a second protein can be, for example, a peptide, a polypeptide, a peptidomimetic or a small organic molecule. Such agents can be useful for modulating the level of phosphorylation of I.kappa.B in a cell, thereby modulating the activity of NF-.kappa.B in the cell and the expression of a gene regulated by NF-.kappa.B.

Summary of Invention Paragraph - BSTX (18):

[0018] The invention also provides methods of identifying proteins that can interact with an I.kappa.B kinase, including with an **IKK** subunit, such proteins which can be a downstream effector of the **IKK** such as a member of the I.kappa.B family of proteins or an upstream activator or a regulatory subunit of an **IKK**. Such proteins that interact with an **IKK complex or the IKK** subunit can be isolated, for example, by coprecipitation with the **IKK** or by using the **IKK** subunit as a ligand, and can be involved, for example, in tissue specific regulation of NF-.kappa.B activation and consequent tissue specific gene expression.

Brief Description of Drawings Paragraph - DRTX (2):

[0019] FIG. 1 shows a nucleotide sequence (SEQ ID NO: 1; lower case letter) and deduced amino acid sequence (SEQ ID NO: 2; upper case letters) of full length human IKK.alpha. subunit of an **IKK complex**. Nucleotide positions are indicated to the right and left of the sequence; the "A" of the ATG encoding the initiator methionine is shown as position 1. Underlined amino acid residues indicate the peptide portions of the protein ("peptide 1" and "peptide 2") that were sequenced and used to design oligonucleotide probes. The asterisk indicates the sequence encoding the STOP codon.

Detail Description Paragraph - DETX (2):

[0022] The present invention provides isolated nucleic acid molecules encoding polypeptide subunits of human serine protein kinase **complex,** the I.kappa.B kinase (**IKK**), which is activated in response to proinflammatory signals and phosphorylates proteins (I.kappa.B's) that bind to and inhibit the activity of NF-.kappa.B transcription factors. For example, the invention provides an isolated nucleic acid molecule (SEQ ID NO: 1) encoding a full length human IKK.alpha. subunit having the amino acid sequence shown as SEQ ID NO: 2 (FIG. 1). In addition, the invention provides an isolated nucleic acid molecule (SEQ ID NO: 14; FIG. 2) encoding a full length human IKK.beta. subunit having the amino acid sequence shown as SEQ ID NO: 15 (FIG. 3).

Detail Description Paragraph - DETX (4):

[0024] IKK.alpha. and IKK.beta. have been designated **IKK** subunits because they are components of an approximately 900 kDa **complex** having I.kappa.B kinase (**IKK**) activity and because they share substantial nucleotide and amino acid sequence homology. As disclosed herein, IKK.alpha. and IKK.beta. are related members of a family of **IKK** catalytic subunits (see FIG. 3). The 900 kDa I.kappa.B kinase **complex** can be isolated in a single step, for example, by immunoprecipitation using an antibody specific for an **IKK** subunit or by using metal ion chelation chromatography methods (see Example IV). A 300 kDa **IKK complex** also can be isolated as disclosed herein and has kinase activity for an I.kappa.B substrate (see Example III).

Detail Description Paragraph - DETX (43):

[0063] The present invention provides an isolated I.kappa.B kinase (**IKK**), including isolated full length **IKK** catalytic subunits. For example, the invention provides an isolated 300 kDa or 900 kDa **complex,** which comprises an IKK.alpha. or an IKK.beta. subunit and has I.kappa.B kinase activity (see Examples I, III and IV). In addition, the invention provides is an isolated human IKK.alpha. catalytic subunit (SEQ ID NO: 2; Example II), which contains a previously undescribed N-terminal amino acid sequence and essentially the C-terminal region of human CHUK (Connelly and Marcu, supra, 1995) and phosphorylates I.kappa.B.alpha. on Ser-32 and Ser-36 and I.kappa.B.beta. on Ser-19 and Ser-23 (DiDonato et al., supra, 1996; see, also, Regnier et al., supra, 1997). The invention also provides an isolated IKK.beta. catalytic subunit (SEQ ID NO: 15; Example III), which shares greater than 50% amino acid sequence identity with IKK.alpha., including conserved homology in the kinase domain, helix-loop-helix domain and leucine zipper domain.

Detail Description Paragraph - DETX (44):

[0064] As used herein, the term "isolated," when used in reference to an I.kappa.B kinase **complex or to an IKK** catalytic subunit of the invention, means

that the **complex** or the subunit is relatively free from contaminating lipids, proteins, nucleic acids or other cellular material normally associated with an **IKK** in a cell. An isolated 900 kDa I.kappa.B kinase **complex** or 300 kDa **complex** can be isolated, for example, by immunoprecipitation using an antibody that binds to an **IKK** catalytic subunit (see Examples III and IV). In addition, an isolated **IKK** subunit can be obtained, for example, by expression of a recombinant nucleic acid molecule such as SEQ ID NO: 1 or SEQ ID NO: 14, or can be isolated from a cell by a method comprising affinity chromatography using ATP or I.kappa.B as ligands (Example I) or using an anti-**IKK** subunit antibody. An isolated **IKK complex or IKK** subunit comprises at least 30% of the material in a sample, generally about 50% or 70% or 90% of a sample, and preferably about 95% or 98% of a sample, as described above with respect to nucleic acids.

Detail Description Paragraph - DETX (52):

[0072] A peptide portion of an **IKK** subunit can comprise the kinase domain of the **IKK** subunit and, therefore, can have the ability to phosphorylate an I.kappa.B protein. For example, a peptide portion of SEQ ID NO: 2 comprising amino acids 15 to 301 has the characteristics of a serine-threonine protein kinase domain (Hanks and Quinn, Meth. Enzymol. 200:38-62 (1991), which is incorporated herein by reference). Such a peptide portion of an **IKK** subunit can be examined for kinase activity by determining that it can phosphorylate I.kappa.B.alpha. at Ser-32 and Ser-36 or I.kappa.B.beta. at Ser-19 and Ser-23, using methods as disclosed herein. In addition, a peptide portion of an **IKK** subunit can comprise an immunogenic amino acid sequence of the polypeptide and, therefore, can be useful for eliciting production of an antibody that can specifically bind the **IKK** subunit or to an **IKK complex** comprising the subunit, particularly to an epitope comprising amino acid residue 30 as shown in SEQ ID NO: 2 or to an epitope of SEQ ID NO: 15, provided said epitope is not present in a CHUK protein. Accordingly, the invention also provides anti-**IKK** antibodies, which specifically bind to an epitope of an **IKK complex, particularly an IKK** catalytic subunit, and to **IKK** subunit binding fragments of such antibodies. In addition, the invention provides cell lines producing anti-**IKK** antibodies or **IKK**-binding fragments of such antibodies.

Detail Description Paragraph - DETX (53):

[0073] As used herein, the term "antibody" is used in its broadest sense to include polyclonal and monoclonal antibodies, as well as antigen binding fragments of such antibodies. With regard to an anti-**IKK** antibody of the invention, the term "antigen" means an **IKK** catalytic subunit protein, polypeptide or peptide portion thereof, or an **IKK complex** comprising an **IKK** catalytic subunit protein, polypeptide or peptide portion thereof. Thus, it should be recognized that, while an anti-**IKK** antibody can bind to and, for example, immunoprecipitate an **IKK complex,** the antibody specifically binds an epitope comprising at least a portion of an **IKK** catalytic subunit. An antibody of the invention also can be used to immunoprecipitate an **IKK** subunit, free of the **IKK complex**.

Detail Description Paragraph - DETX (56):

[0076] An anti-**IKK** antibody of the invention can be raised using an isolated **IKK** subunit or a peptide portion thereof and can bind to a free, uncomplexed form of **IKK** subunit or can bind to **IKK** subunit when it is associated with a 300 kDa or 900 kDa **IKK complex**. In addition, an anti-**IKK** antibody of the invention can be raised against an isolated 300 kDa or 900 kDa I.kappa.B kinase **complex,** which can be obtained as disclosed herein. For convenience, an antibody of the invention is referred to generally herein as an "anti-I.kappa.B kinase antibody" or an "anti-**IKK** antibody." However, the skilled recognize that the various antibodies of the invention will have unique antigenic specificities, for example, for a free or complexed **IKK** subunit, or both, or for a 300 kDa or 900 kDa I.kappa.B kinase **complex,** or both.

Detail Description Paragraph - DETX (58):

[0078] Particularly useful antibodies of the invention include antibodies that bind with the free, but not the complexed, form of an **IKK** subunit or, alternatively, with the complexed, but not free, form of an **IKK** subunit. Antibodies of the invention also include antibodies that bind with the 300 kDa I.kappa.B kinase **complex** or the 900 kDa I.kappa.B kinase **complex** or both. It should be recognized, however, that an antibody specific for the 300 kDa or 900 kDa I.kappa.B kinase **complex** need not recognize an **IKK** subunit epitope in order to be encompassed within the claimed invention, since, prior to the present disclosure, the 300 kDa and 900 kDa **IKK** complexes were not known (see DiDonato et al., Nature 388:548-554 (1997)).

Detail Description Paragraph - DETX (59):

[0079] Antibodies of the invention that bind to an activated **IKK** but not to an inactive **IKK,** and, conversely, those that bind to an inactive form of the kinase but not to the activated form also are particularly useful. For example, an **IKK** can be activated by phosphorylation of an **IKK** subunit and, therefore, an antibody that recognizes the phosphorylated form of the **IKK,** but that does not bind to the unphosphorylated form can be obtained. In addition, **IKK** can be activated by release of a regulatory subunit and, therefore, an antibody that recognizes a form of the **IKK complex** that is not bound to the regulatory subunit can be obtained. Such antibodies are useful for identifying the presence of active **IKK** in a cell.

Detail Description Paragraph - DETX (61):

[0081] A kit incorporating an anti-**IKK** antibody, which can be specific for the active or inactive form of I.kappa.B kinase or can bind to an **IKK complex or to an IKK** subunit, regardless of the activity state, can be particularly useful. Such a kit can contain, in addition to an anti-**IKK** antibody, a reaction cocktail that provides the proper conditions for performing the assay, control samples that contain known amounts of an **IKK or IKK** subunit and, if desired, a second antibody specific for the anti-**IKK** antibody. Such an assay

also should include a simple method for detecting the presence or amount of an **IKK or an IKK** subunit in a sample that is bound to the anti-**IKK** antibody.

Detail Description Paragraph - DETX (64):

[0084] Methods for raising polyclonal antibodies, for example, in a rabbit, goat, mouse or other mammal, are well known in the art (see Example V). In addition, monoclonal antibodies can be obtained using methods that are well known and routine in the art (Harlow and Lane, supra, 1988). Essentially, spleen cells from a mouse immunized with an **IKK complex or an IKK** subunit or peptide portion thereof can be fused to an appropriate myeloma cell line such as SP/02 myeloma cells to produce hybridoma cells. Cloned hybridoma cell lines can be screened using a labeled **IKK** subunit to identify clones that secrete anti-**IKK** monoclonal antibodies. Hybridomas expressing anti-**IKK** monoclonal antibodies having a desirable specificity and affinity can be isolated and utilized as a continuous source of the antibodies, which are useful, for example, for preparing standardized kits as described above. Similarly, a recombinant phage that expresses, for example, a single chain anti-**IKK** also provides a monoclonal antibody that can used for preparing standardized kits.

Detail Description Paragraph - DETX (66):

[0086] The present invention further provides methods of identifying an agent that can alter the association of an **IKK** catalytic subunit with a second protein, which can be an upstream activator, a downstream effector such as I.kappa.B, an interacting regulatory protein of the **IKK** subunit, or an interacting subunit associated with the 300 kDa or 900 kDa I.kappa.B kinase **complex**. As used herein, the term "associate" or "association," when used in reference to an **IKK** subunit and a second protein means that the **IKK** subunit and the second protein have a binding affinity for each other such that they form a bound **complex** in vivo or in vitro, including in a cell in culture or in a reaction comprising substantially purified reagents. For convenience, the term "bind" or "interact" is used interchangeably with the term "associate."

Detail Description Paragraph - DETX (67):

[0087] The affinity of binding of an **IKK** subunit and a second protein such as an I.kappa.B or another **IKK** subunit or other subunit present in an **IKK complex** is characterized in that it is sufficiently specific such that a bound **complex** can form in vivo in a cell or can form in vitro under appropriate conditions as disclosed herein. The formation or dissociation of a bound **complex** can be identified, for example, using the two hybrid assay or demonstrating coimmunoprecipitation of the second protein with the **IKK** subunit, as disclosed herein, or using other well known methods such as equilibrium dialysis. Methods for distinguishing the specific association of an **IKK** subunit and a second protein from nonspecific binding to the **IKK** subunit are known in the art and, generally, include performing the appropriate control experiments to demonstrate the absence of nonspecific protein binding.

Detail Description Paragraph - DETX (68):

[0088] As used herein, the term "second protein" refers to a protein that specifically associates with an **IKK** subunit ("first protein"). Such a second protein is exemplified herein by I.kappa.B proteins, including I.kappa.B.alpha. and I.kappa.B.beta., which are substrates for I.kappa.B kinase activity and are downstream of the I.kappa.B kinase in a signal transduction pathway that results in the regulated expression of a gene. In addition, such second proteins are exemplified by the proteins that, together with the **IKK** subunits, form a 300 kDa or 900 kDa I.kappa.B kinase **complex,** which coimmunoprecipitates using an anti-**IKK** antibody (see Example IV). Furthermore, since **IKK** subunits such as IKK.alpha. and IKK.beta. interact with each other to form homodimers or heterodimers, a second protein also can be a second **IKK** subunit, which can be the same as or different from the "first" protein.

Detail Description Paragraph - DETX (70):

[0090] In addition, a second protein can be a protein that is upstream of I.kappa.B kinase in a signal transduction pathway and associates with the **IKK complex, particularly with an IKK** catalytic subunit of the **IKK complex**. Such a second protein, which can be an upstream activator of the I.kappa.B kinase, can be identified using routine methods for identifying protein-protein interactions as disclosed herein. Such second proteins can be, for example, MEKK1 or PKR or CKII, each of which has been reported to be involved in a pathway leading to phosphorylation of I.kappa.B and activation of NF-.kappa.B, but neither of which has the characteristics expected of the common I.kappa.B kinase present at the point where the various NF-.kappa.B activation pathways converge (see, for example, Lee et al., supra, 1997), or can be the NF-.kappa.B-inducing kinase (NIK), which reportedly is upstream from **IKK** in an NF-.kappa.B activation pathway (Regnier et al., supra, 1997; Malinin et al., Nature 385:540-544 (1997)).

Detail Description Paragraph - DETX (71):

[0091] A second protein also can be a regulatory protein, which associates with an **IKK** catalytic subunit in an **IKK complex,** either constitutively as part of a 300 kDa or 900 kDa **complex** or in response to activation of a pathway leading to **IKK** activation. Such a regulatory protein can inhibit or activate **IKK** activity depending, for example, on whether the regulatory protein is associated with **IKK** and whether the regulatory protein associates with an **IKK** catalytic subunit in a free form or as part of an **IKK complex**. The regulatory protein also can be important for "docking" a catalytic **IKK** subunit to its substrate. The ability of a regulatory protein to associate with or dissociate from an **IKK** subunit or **IKK complex** can depend, for example, on the relative phosphorylation state of the regulatory protein. It is recognized that an upstream activator of **IKK** also can interact with such a regulatory protein, thereby indirectly inhibiting or activating the **IKK**.

Detail Description Paragraph - DETX (72):

[0092] As disclosed herein, two copurifying proteins were isolated by ATP and I.kappa.B affinity chromatography and identified by SDS-PAGE (Example I). Partial amino acid sequences were determined and cDNA molecules encoding the proteins were obtained (see Examples I, II and III). One of the proteins has an apparent molecular mass of 85 kDa. Expression in a cell of a cDNA molecule encoding the 85 kDa protein resulted in increased NF-.kappa.B activity following cytokine induction as compared to control cells, whereas expression of the antisense of this cDNA decreased the basal NF-.kappa.B activity in the cells and prevented cytokine induction of NF-.kappa.B activity. Immunoprecipitation of the 85 kDa protein resulted in isolation of the **IKK complex,** the kinase activity of which was stimulated rapidly in response to TNF or to IL-1. Based on these functional analyses, the 85 kDa protein was determined to be a component of the 900 kDa I.kappa.B kinase **complex** and has been designated IKK.alpha. (SEQ ID NO: 2). The second protein, which copurified with the 85 kDa I.kappa.B kinase, has an apparent molecular mass of 87 kDa and shares greater than 50% amino acid sequence identity with IKK.alpha. and has been designated IKK.beta. (SEQ ID NO: 15).

Detail Description Paragraph - DETX (74):

[0094] A screening assay of the invention provides a means to identify an agent that alters the association of an **IKK complex or an IKK** catalytic subunit with a second protein such as the regulatory subunits discussed above. As used herein, the term "modulate" or "alter" when used in reference to the association of an **IKK** and a second protein, means that the affinity of the association is increased or decreased with respect to a steady state, control level of association, i.e., in the absence of an agent. Agents that can alter the association of an **IKK** with a second protein can be useful for modulating the level of phosphorylation of I.kappa.B in a cell, which, in turn, modulates the activity of NF-.kappa.B in the cell and the expression of a gene regulated by NF-.kappa.B. Such an agent can be, for example, an anti-idiotypic antibody as described above, which can inhibit the association of an **IKK** and I.kappa.B. A peptide portion of I.kappa.B.alpha. comprising amino acids 32 to 36, but containing substitutions for Ser-32 and Ser-36, is another example of such an agent, since the peptide can compete with I.kappa.B.alpha. binding to **IKK,** as is the corresponding peptide of I.kappa.B.beta..

Detail Description Paragraph - DETX (75):

[0095] A screening assay of the invention also is useful for identifying agents that directly alter the activity of an **IKK**. While such an agent can act, for example, by altering the association of an **IKK complex or IKK** catalytic subunit with a second protein, the agent also can act directly as a specific activator or inhibitor of **IKK** activity. Specific protein kinase inhibitors include, for example, staurosporin, the heat stable inhibitor of cAMP-dependent protein kinase, and the MLCK inhibitor, which are known in the art and commercially available. A library of molecules based, generally, on such inhibitors or on ATP or adenosine can be screened using an assay of the

invention to obtain agents that desirably modulate the activity of an **IKK complex or an IKK** subunit.

Detail Description Paragraph - DETX (77):

[0097] A screening assay of the invention is particularly useful to identify, from among a diverse population of molecules, those agents that modulate the association of an **IKK complex or an IKK** catalytic subunit and another protein (referred to herein as a "second protein") or that directly alter the activity of **IKK**. Methods for producing libraries containing diverse populations of molecules, including chemical or biological molecules such as simple or **complex** organic molecules, peptides, proteins, peptidomimetics, glycoproteins, lipoproteins, polynucleotides, and the like, are well known in the art (Huse, U.S. Pat. No. 5,264,563, issued Nov. 23, 1993; Blondelle et al., Trends Anal. Chem. 14:83-92 (1995); York et al., Science 274:1520-1522 (1996); Gold et al., Proc. Natl. Acad. Sci., USA 94:59-64 (1997); Gold, U.S. Pat. No. 5,270,163, issued Dec. 14, 1993). Such libraries also can be obtained from commercial sources.

Detail Description Paragraph - DETX (79):

[0099] A drug screening assay of the invention utilizes an **IKK complex,** which can be isolated as disclosed herein; or an **IKK** subunit, which can be expressed, for example, from a nucleic acid molecule encoding the amino acid sequence shown in SEQ ID NO: 2 or in SEQ ID NO: 15; or can be purified as disclosed herein; or can utilize an **IKK** subunit fusion protein such as an IKK.alpha.-glutathione-S-transferase (GST) or IKK.beta.-histidine.sub.6 (HIS6) fusion protein, wherein the GST or HIS6 is linked to the **IKK** subunit and comprises a tag (see Example VI). The **IKK or IKK** subunit fusion protein is characterized, in part, by having an affinity for a solid substrate as well as having the ability to specifically associate with an appropriate second protein such as an I.kappa.B protein. For example, when an **IKK** catalytic subunit is used in a screening assay, the solid substrate can contain a covalently attached anti-**IKK** antibody, provided that the antibody binds the **IKK** subunit without interfering with the ability of the **IKK** subunit to associate with the second protein. Where an IKK.alpha.-GST fusion protein, for example, is used in such a screening assay, the solid substrate can contain covalently attached glutathione, which is bound by the GST tag component of the fusion protein. If desired, the **IKK** subunit or **IKK** subunit fusion protein can be part of an **IKK complex** in a drug screening assay of the invention.

Detail Description Paragraph - DETX (80):

[0100] A drug screening assay to identify an agent that alters the association of an **IKK complex or an IKK** subunit and a second protein can be performed by allowing, for example, the **IKK complex or IKK** subunit, which can be a fusion protein, to bind to the solid support, then adding the second protein, which can be an I.kappa.B such as I.kappa.B.alpha., and an agent to be tested, under conditions suitable for the association of the **IKK** and

I.kappa.B.alpha. in the absence of a drug (see Example VI). As appropriate, the **IKK** can be activated or inactivated as disclosed herein and, typically, the **IKK** or the second protein is detectably labeled so as to facilitate identification of the association. Control reactions, which contain or lack either, the **IKK** component, or the I.kappa.B protein, or the agent, or which substitute the I.kappa.B protein with a second protein that is known not to associate specifically with the **IKK,** also are performed. Following incubation of the reaction mixture, the amount of I.kappa.B.alpha. specifically bound to the **IKK** in the presence of an agent can be determined and compared to the amount of binding in the absence of the agent so that agents that modulate the association can be identified.

Detail Description Paragraph - DETX (81):

[0101] An **IKK** subunit such as IKK.alpha. or IKK.beta. used in a screening assay can be detectably labeled with a radionuclide, a fluorescent label, an enzyme, a peptide epitope or other such moiety, which facilitates a determination of the amount of association in a reaction. By comparing the amount of specific binding of an **IKK** subunit or an **IKK complex** and I.kappa.B in the presence of an agent as compared to the control level of binding, an agent that increases or decreases the binding of the **IKK** and the I.kappa.B can be identified. In comparison, where a drug screening assay is used to identify an agent that alters the activity of an **IKK,** the detectable label can be, for example, .gamma.-.sup.32P-ATP, and the amount of .sup.32P-I.kappa.B can be detected as a measure of **IKK** activity. Thus, the drug screening assay provides a rapid and simple method for selecting agents that desirably alter the association of an **IKK** and a second protein such as an I.kappa.B or for altering the activity of an **IKK**. Such agents can be useful, for example, for modulating the activity of NF-.kappa.B in a cell and, therefore, can be useful as medicaments for the treatment of a pathology due, at least in part, to aberrant NF-.kappa.B activity.

Detail Description Paragraph - DETX (84):

[0104] The invention also provides a method of obtaining an isolated **IKK complex or an IKK** catalytic subunit. For example, a 300 kDa or a 900 kDa **IKK complex,** comprising an IKK.alpha. subunit can be isolated from a sample by immunoprecipitation using an anti-IKK.alpha. antibody or by tagging the IKK.alpha. and using an antibody specific for the tag (see Examples III and IV). In addition, an **IKK** catalytic subunit can be isolated from a sample by 1) incubating the sample containing the **IKK** subunit with ATP, which is immobilized on a matrix, under conditions suitable for binding of the **IKK** subunit to the ATP; 2) obtaining from the immobilized ATP a fraction of the sample containing the **IKK** subunit; 3) incubating the fraction containing the **IKK** subunit with an I.kappa.B, which is immobilized on a matrix, under conditions suitable for binding of the **IKK** subunit to the I.kappa.B; and 4) obtaining from the immobilized I.kappa.B an isolated **IKK** catalytic subunit. Such a method of isolating an **IKK** subunit is exemplified herein by the use of ATP affinity chromatography and I.kappa.B.alpha. affinity chromatography to isolate IKK.alpha. or IKK.beta. from a sample of HeLa cells (see Example I).

Detail Description Paragraph - DETX (85):

[0105] The skilled artisan will recognize that a ligand such as ATP or an I.kappa.B or an anti-**IKK** antibody also can be immobilized on various other matrices, including, for example, on magnetic beads, which provide a rapid and simple method of obtaining a fraction containing an ATP-or an I.kappa.B-bound **IKK complex or IKK** subunit or an anti-I.kappa.B kinase-bound **IKK** from the remainder of the sample. Methods for immobilizing a ligand such as ATP or an I.kappa.B or an antibody are well known in the art (Haystead et al., Eur. J. Biochem. 214:459-467 (1993), which is incorporated herein by reference; see, also, Hermanson, supra, 1996). Similarly, the artisan will recognize that a sample containing an **IKK complex or an IKK** subunit can be a cell, tissue or organ sample, which is obtained from an animal, including a mammal such as a human, and prepared as a lysate; or can be a bacterial, insect, yeast or mammalian cell lysate, in which an **IKK** catalytic subunit is expressed from a recombinant nucleic acid molecule. As disclosed herein, a recombinantly expressed IKK.alpha. or IKK.beta. such as a tagged IKK.alpha. or IKK.beta. associates into an active 300 kDa and 900 kDa **IKK complex** (see Examples III and IV).

Detail Description Paragraph - DETX (86):

[0106] The invention also provides a method of identifying a second protein that associates with an **IKK complex, particularly with an IKK** subunit. A transcription activation assay such as the yeast two hybrid system is particularly useful for the identification of protein-protein interactions (Fields and Song, Nature 340:245-246 (1989), which is incorporated herein by reference). In addition, the two hybrid assay is useful for the manipulation of protein-protein interaction and, therefore, also is useful in a screening assay to identify agents that modulate the specific interaction.

Detail Description Paragraph - DETX (89):

[0109] One adaptation of the transcription activation assay, the yeast two hybrid system, uses S. cerevisiae as a host cell for vectors that express the hybrid proteins. For example, a yeast host cell containing a reporter lacZ gene linked to a LexA operator sequence can be used to identify specific interactions between an **IKK** subunit and a second protein, where the DNA-binding domain is the LexA binding domain, which binds the LexA promoter, and the trans-activation domain is the B42 acidic region. When the LexA domain is bridged to the B42 transactivation domain through the interaction of the **IKK** subunit with a second protein, which can be expressed, for example, from a cDNA library, transcription of the reporter lacZ gene is activated. In this way, proteins that interact with the **IKK** subunit can be identified and their role in a signal transduction pathway mediated by the **IKK** can be elucidated. Such second proteins can include additional subunits comprising the 300 kDa or 900 kDa **IKK complex**.

Detail Description Paragraph - DETX (92):

[0112] An agent that alters the catalytic activity of an **IKK** or that alters the association of an **IKK** subunit or **IKK complex** and a second protein such as an I.kappa.B or an **IKK** regulatory subunit or an upstream activator of an **IKK** can be useful as a drug to reduce the severity of a pathology characterized by aberrant NF-.kappa.B activity. For example, a drug that increases the activity of an **IKK** or that increases the affinity of an **IKK** catalytic subunit and I.kappa.B.alpha. can increase the amount of I.kappa.B.alpha. phosphorylated on Ser-32 or Ser-36 and, therefore, increase the amount of active NF-.kappa.B and the expression of a gene regulated by NF-.kappa.B, since the drug will increase the level of phosphorylated I.kappa.B.alpha. in the cell, thereby allowing NF-.kappa.B translocation to the nucleus. In contrast, a drug that decreases or inhibits the catalytic activity of an **IKK** or the association of an **IKK** catalytic subunit and I.kappa.B.alpha. can be useful where it is desirable to decrease the level of active NF-.kappa.B in a cell and the expression of a gene induced by activated NF-.kappa.B. It should be recognized that an antisense **IKK** subunit molecule of the invention also can be used to decrease **IKK** activity in a cell by reducing or inhibiting expression of the **IKK** subunit or by reducing or inhibiting its responsiveness to an inducing agent such as TNF.alpha., Il-1 or phorbol ester (see Example II). Accordingly, the invention also provides methods of treating an individual suffering from a pathology characterized by aberrant NF-.kappa.B activity by administering to the individual an agent that modulates the catalytic activity of an **IKK** or that alters the association of an **IKK** subunit and a second protein such as I.kappa.B or a subunit of a 300 kDa or 900 kDa **IKK complex** that interacts with the **IKK** subunit.

Detail Description Paragraph - DETX (94):

[0114] Glucocorticoids are potent anti-inflammatory and immunosuppressive agents that are used clinically to treat various pathologic conditions, including autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosis and asthma. Glucocorticoids suppress the immune and inflammatory responses, at least in part, by increasing the rate of I.kappa.B.alpha. synthesis, resulting in increased cellular levels of I.kappa.B.alpha., which bind to and inactivate NF-.kappa.B (Scheinman et al., Science 270:283-286 (1995); Auphan et al., Science 270:286-290 (1995)). Thus, glucocorticoids suppress NF-.kappa.B mediated expression of genes encoding, for example, cytokines, thereby suppressing the immune, inflammatory and acute phase responses. However, glucocorticoids and glucocorticoid-like steroids also are produced physiologically and are required for normal growth and development. Unfortunately, prolonged treatment of an individual with higher than physiological amounts of glucocorticoids produces clinically undesirable side effects. Thus, the use of an agent that alters the activity of an **IKK** or that alters the association of an **IKK complex or IKK** subunit and a second protein, as identified using a method of the invention, can provide a means for selectively altering NF-.kappa.B activity without producing some of the undesirable side effects associated with glucocorticoid treatment.

Detail Description Paragraph - DETX (97):

[0117] In addition, the decreased level of NF-.kappa.B activity may allow the virus-infected cell to undergo apoptosis, resulting in a decrease in the viral load in the individual. As such, it can be particularly useful to treat virus-infected cells ex vivo with an agent identified using a method of the invention. For example, peripheral blood mononuclear cells (PBMCs) can be collected from an HIV-1 infected individual and treated in culture with an agent that decreases the activity of an **IKK** or alters the association of an **IKK complex or an IKK** catalytic subunit with an I.kappa.B. Such a treatment can be useful to purge the PBMCs of the virus-infected cells by allowing apoptosis to proceed. The purged population of PBMCs then can be expanded, if desired, and readministered to the individual.

Detail Description Paragraph - DETX (98):

[0118] Rel/NF-.kappa.B proteins also are involved in a number of different types of cancer. For example, the adhesion of cancer cells to endothelial cells is increased due to treatment of the cancer cells with IL-1, suggesting that NF-.kappa.B induced the expression of cell adhesion molecules, which mediated adherence of the tumor cells to the endothelial cells; agents such as aspirin, which decrease NF-.kappa.B activity, blocked the adhesion by inhibiting expression of the cell adhesion molecules (Tozawa et al., Cancer Res. 55:4162-4167 (1995)). These results indicate that an agent that decreases the activity of an **IKK** or that decrease the association of an **IKK** and I.kappa.B or of an **IKK** subunit and a second protein, for example, a second protein present in an **IKK complex,** can be useful for reducing the likelihood of metastasis of a tumor in an individual.

Detail Description Paragraph - DETX (102):

Identification and Characterization of a Human I.kappa.B Kinase **Complex And IKK** Subunits

Detail Description Paragraph - DETX (108):

[0126] B. Purification of **IKK complex and IKK** subunits:

Detail Description Paragraph - DETX (122):

[0140] Since the 85 kDa IKK.alpha. band identified by the kinase assay following the above procedure contained only about 10% of the total purified protein, three additional criteria were used to confirm that the identified band was an intrinsic component of the **IKK complex**.

Detail Description Paragraph - DETX (163):

[0177] Similarly to the purified **IKK complex and the complex** associated with IKK.alpha., the IKK.beta. immune **complex** phosphorylated wt I.kappa.B.alpha. and I.kappa.B.beta., but not mutants in which the inducible phosphorylation sites (Ser-32 and Ser-36 for I.kappa.B.alpha. and Ser-19 and Ser-23 for I.kappa.B.alpha.) were replaced with either alanines or threonines. However, a low level of residual phosphorylation of full length I.kappa.B.alpha.(A32/A36) was observed due to phosphorylation of sites in the C-terminal portion of the protein (DiDonato et al., supra, 1997). Single substitution mutants, I.kappa.B.alpha.(A32) and I.kappa.B(A36), were phosphorylated almost as efficiently as wt I.kappa.B.alpha., indicating that IKK.beta.-associated **IKK** activity can phosphorylate I.kappa.B.alpha. at both Ser-32 and Ser-36.

Detail Description Paragraph - DETX (166):

[0180] As shown in Example I, IKK.alpha. and IKK.beta. copurified in about a 1:1 ratio through several chromatographic steps, suggesting that the two proteins interact with each other. The ability of the **IKK** subunits to interact in a functional **complex** and the effect of each subunit on the activity of the other subunit was examined using 293 cells transfected with expression vectors encoding Flag(M2)-IKK.alpha. or M2-IKK.alpha. and HA-IKK.alpha., either alone or in combination (see Hopp et al., BioTechnology 6:1204-1210 (1988)). After 24 hr, samples of the cells were stimulated with TNF, lysates were prepared from stimulated and unstimulated cells, and one portion of the lysates was precipitated with anti-Flag antibodies (Eastman Kodak Co.; New Haven Conn.) and another portion was precipitated with anti-HA antibodies. The **IKK** activity associated with the different immune complexes and their content of IKK.alpha. and IKK.beta. were measured.

Detail Description Paragraph - DETX (169):

[0183] The HA-IKK.alpha.-associated **IKK** had a low level of basal specific activity, whereas expression of HA-IKK.beta. resulted in high basal specific activity that was increased when higher amounts of HA-IKK.beta. were expressed. However, the specific **IKK** activity associated with either IKK.alpha. or IKK.beta. isolated from TNF-stimulated cells was very similar and was not considerably affected by their expression level. These results indicate that titration of a negative regulator or formation of a constitutively active **IKK complex** can occur due to overexpression of IKK.beta..

Detail Description Paragraph - DETX (172):

[0186] Since the results described above revealed that HA-IKK.beta. associates with endogenous IKK.alpha. to generate a functional cytokine-regulated **IKK complex,** this association was examined further by transfecting HeLa cells with either empty expression vector or small amounts (1 .mu.g/60 mm plate) of either HA-IKK.alpha. or HA-IKK.beta. vectors. After 24 hr, samples of the transfected cell populations were stimulated with 20 ng/ml TNF for 5 min, then cell lysates were prepared and separated by gel filtration

on a SUPEROSE 6 column. One portion of each column fraction was immunoprecipitated with a polyclonal antibody specific for IKK.alpha. and assayed for IKK.alpha.-associated **IKK** activity, while a second portion was precipitated with anti-HA antibody and examined for HA-IKK.beta.- or HA-IKK.alpha.-associated **IKK** activity. Relative specific activity was determined by immunoprecipitating the complexes, separating the proteins by SDS-PAGE, blotting the proteins onto IMOBILON membranes (Millipore; Bedford Mass.), immunoblotting with anti-HA antibody and quantitating the levels of I.kappa.B phosphorylation and HA-tagged proteins by phosphoimaging. The results demonstrated that endogenous IKK.alpha.-associated **IKK** activity exists as two complexes, a larger **complex** of approximately 900 kDa and a smaller one of approximately 300 kDa. Stimulation with TNF increased the **IKK** activity of both complexes, although the extent of increase was considerably greater for the 900 kDa **complex**.

Detail Description Paragraph - DETX (173):

[0187] HA-IKK.beta.-associated **IKK** activity had exactly the same distribution as the IKK.alpha.-associated activity, eluting at 900 kDa and 300 kDa and, again, the extent of TNF responsiveness was considerably greater for the 900 kDa **complex**. Comparison to the IKK.alpha.-associated activity in cells transfected with the empty vector indicated that HA-IKK.beta. expression produced a modest, approximately 2-fold increase in the relative amount of **IKK** activity associated with the smaller 300 kDa **complex**. These results indicate that the 300 kDa **IKK complex,** like the 900 kDa **complex,** contains both IKK.alpha. and IKK.beta.. However, the 300 kDa lacks other subunits present in the 900 kDa **complex**. When IKK.beta. was overexpressed, the relative amount of the smaller **complex** increased, indicating that some of the subunits that are unique to the larger **complex** are present in a limited amount.

Detail Description Paragraph - DETX (206):

[0214] Alternatively, the labeled I.kappa.B or other appropriate second protein can be added to the immobilized **IKK** subunit and allowed to associate, then the agent can be added. Such a method allows the identification of agents that can induce the dissociation of a bound **complex** comprising the **IKK** subunit and I.kappa.B. Similarly, a screening assay of the invention can be performed using the 900 kDa **IKK complex,** comprising an **IKK** subunit.

Claims Text - CLTX (40):

39. The method of claim 34, wherein said antibody specifically binds an **IKK complex**.

PGPUB-DOCUMENT-NUMBER: 20020042499

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020042499 A1

TITLE: Novel inhibitor of the inflammatory response induced by TNF-alpha and IL-1

PUBLICATION-DATE: April 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Lin, Xin	San Francisco	CA	US	
Gelezuinas, Romas	San Francisco	CA	US	

APPL-NO: 09/ 871889

DATE FILED: June 1, 2001

RELATED-US-APPL-DATA:

child 09871889 A1 20010601

parent division-of 09257703 19990225 US PATENTED

non-provisional-of-provisional 60076299 19980227 US

US-CL-CURRENT: 530/351, 435/194

ABSTRACT:

The present invention provides the molecular basis for cytokine induction of NF-.kappa.B-dependent immune and inflammatory responses, emphasizing a role for both NIK-NIK and NIK-IKK protein-protein interactions. A relatively small region of NIK selectively impairs the NIK-IKK interaction. The present invention provides a novel and highly specific method for modulating NF-.kappa.B-dependent immune, inflammatory, and anti-apoptotic responses, based on interruption of the critical protein-protein interaction of NIK and IKK. The present invention provides methods for inhibiting NF-.kappa.B-dependent gene expression, using mutant NIK proteins. One embodiment of the present invention provides kinase-deficient NIK mutant proteins that inhibit activation of IKK. Another embodiment of the invention provides N-terminus NIK mutant proteins that bind IKK, thus inhibiting NIK/IKK interaction.

[0001] This application claims priority of U.S. Serial No. 60/076,299, filed Feb. 27, 1998.

---------- KWIC ---------

Detail Description Paragraph - DETX (9):

[0025] The term "NIK/**IKK**" interaction used herein is defined as NIK/**IKK** protein binding, a NIK/**IKK** protein **complex** and NIK/**IKK** protein assembly.

Detail Description Paragraph - DETX (10):

[0026] The term "inhibition of NIK/**IKK** interaction" used herein is defined as inhibiting either the formation of a NIK/**IKK** protein **complex** or disruption of a formed NIK/**IKK** protein **complex**.

Detail Description Paragraph - DETX (12):

[0028] The term "NF-.kappa.B-dependent gene expression" used herein is defined as those immune and inflammatory genes that are under the regulatory control of the .kappa.B-enhancer. In most cells, NF-.kappa.B exists in a latent state in the cytoplasm bound to inhibitory proteins, collectively called I.kappa.B, that mask the nuclear localization signal thereby preventing nuclear translocation. The latent form of NF-.kappa.B can be induced by cytokines, such as TNF.alpha. and IL-1. Both TNF.alpha. and IL-1 signaling leads to sequential phosphorylation and activation of a series of proteins involved in a cascade pathway that requires NIK/**IKK** protein interaction and **IKK** activation, that in turn leads to phosphorylation and degradation of I.kappa.B. Degradation of the I.kappa.B inhibitor unmasks the nuclear localization signal of the NF-.kappa.B **complex** allowing its rapid translocation into the nucleus where it engages cognate .kappa.B-enhancer elements and activates the transcription of various NF-.kappa.B-dependent genes involved in inflammation and immune response.

Detail Description Paragraph - DETX (21):

[0037] One embodiment of the invention provides a method for inhibiting activation of NF-.kappa.B-dependent gene expression by inhibiting NIK/**IKK** interaction, by contacting an **IKK** protein with a catalytically inactive NIK protein that continues to interact with and bind to **IKK**. The inhibition of the NIK/**IKK** interaction includes inhibiting the formation of a NIK/**IKK complex,** or disruption of a formed NIK/**IKK complex**. One particular embodiment of the present invention provides a method for inhibiting activation of NF-.kappa.B-dependent gene expression by inhibiting wild type NIK/**IKK** interaction, by contacting the wild type **IKK**/NIK protein **complex** with a catalytically inactive or mutant NIK protein, such that the mutant NIK competes with the wild type NIK for assembly with the **IKK,** thereby forming an inactive NIK/**IKK** protein **complex**. One embodiment provides using an N-terminus deletion mutant NIK protein, such as N735-947, to inhibit NIK/**IKK** interaction. These N-terminus mutant NIK proteins bind to **IKK** protein and inhibit wild type NIK/**IKK** interaction, thereby inhibiting activation of NF-.kappa.B-dependent gene expression. The methods of the present invention are not limited to using

only the N-terminus deletion mutant NIK protein N735-947, as the present invention also provides for use of other N-terminus deletion mutant NIK proteins and other types of NIK mutant proteins that can interact with an **IKK** protein to inhibit activation of NF-.kappa.B-dependent gene expression.

Detail Description Paragraph - DETX (115):

[0122] Zandi, E., D. M. Rothwarf, M. Delhase, M. Hayakawa and M. Karin. 1997. The IkappaB kinase **complex (IKK)** contains two kinase subunits, IKKalpha and IKKbeta, necessary for IkappaB phosphorylation and NF-kappaB activation. Cell 91:243-52

Claims Text - CLTX (5):

4. A method for inhibiting a first NIK/**IKK** protein interaction, the method comprising: contacting the first NIK/**IKK** protein **complex** with a mutant NIK protein that competes with the NIK of the first NIK/**IKK complex** so as to form a second NIK/**IKK** protein **complex,** thereby inhibiting the first NIK/**IKK** protein interaction.

Claims Text - CLTX (11):

10. A method for inhibiting activation of an endogenous **IKK** protein, comprising: contacting the endogenous **IKK** protein with a kinase deficient mutant NIK protein so as to form a **complex,** thereby inhibiting activation of the endogenous **IKK** protein.

Claims Text - CLTX (12):

11. A method for inhibiting phosphorylation of an endogenous **IKK** protein, the method comprising: contacting the endogenous **IKK** protein with a kinase deficient mutant NIK protein so as to from a **complex,** thereby inhibiting phosphorylation of the endogenous **IKK** protein.

PGPUB-DOCUMENT-NUMBER: 20020042083

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020042083 A1

TITLE: Ubiquitin ligase assay

PUBLICATION-DATE: April 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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APPL-NO: 09/ 826312

DATE FILED: April 3, 2001

RELATED-US-APPL-DATA:

child 09826312 A1 20010403

parent continuation-in-part-of 09542497 20000403 US PENDING

US-CL-CURRENT: 435/7.9

ABSTRACT:

The invention relates to assays for measuring ubiquitin ligase activity and for identifying modulators of ubiquitin ligase enzymes.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (8):

[0006] Some E3 ubiquitin ligases are known to have a single subunit responsible for the ligase activity. Such E3 ligases that have been characterized include the HECT (homologous to E6-AP carboxy terminus) domain proteins, represented by the mammalian E6AP-E6 **complex** which functions as a ubiquitin ligase for the tumor suppressor p53 and which is activated by papillomavirus in cervical cancer (Huang et al., Science 286:1321-26 (1999)). Single subunit ubiquitin ligases having a RING domain include Mdm2, which has also been shown to act as a ubiquitin ligase for p53, as well as Mdm2 itself. Other RING domain, single subunit E3 ligases include: TRAF6, involved in **IKK** activation; Cb1, which targets insulin and EGF; Sina/Siah, which targets DCC; Itchy, which is involved in haematopoesis (B, T and mast cells); and IAP,

involved with inhibitors of apoptosis.

PGPUB-DOCUMENT-NUMBER: 20010053351

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010053351 A1

TITLE: Inhibition of GSK-3 beta

PUBLICATION-DATE: December 20, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hoeflich, Klaus	Toronto		CA	
Woodgett, James	Toronto		CA	
Luo, Juan	Toronto		CA	

APPL-NO: 09/ 747552

DATE FILED: December 22, 2000

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60172064 19991223 US

US-CL-CURRENT: 424/85.1, 514/1

ABSTRACT:

The activity of NF-.kappa.B is modulated through the effects of GSK-3 on NF-.kappa.B activity. Inhibition or down-regulation of GSK-3 results in decreased NF-.kappa.B activity. Inappropriate activation of NF-.kappa.B has been linked to inflammation and hyperproliferative disorders. Development of modulatory strategies provide a novel therapeutic tool for the treatment or prevention of various diseases. Methods are also provided for enhanced killing of tumor cells through the sensitization action of GSK-3 inhibition, when administered in conjunction with apoptosis inducing ligands of TNFR1. Transgenic animals defective in GSK-3 function are also provided.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (7):

[0006] Tumor necrosis factor (TNF)-.alpha. has been shown to exert cytotoxic or cytostatic effects on tumor cells, but susceptibility to TNF-.alpha. varies among different types of cells. Activation of the type-1 TNF receptor (TNFR1) induces the formation of a signalling **complex** that contains TNF-receptor-associated-factor 2 (TRAF2), which binds NIK, a MAP kinase kinase kinase. Phosphorylation of the **IKK** component of the I.kappa.B kinase **complex** by NIK targets I-.kappa.B for degradation and induces

NF-.kappa.B activation. An active NF-.kappa.B **complex,** such as the p50-p65 heterodimer, plays a crucial role in the progression of cell cycle in some malignancies. In some cases, refractoriness to TNF-.alpha. treatment can be prevented by inhibiting NF-.kappa.B activation (Otsuka et al. (1999) Cancer Research 59(7):4446-52).

PGPUB-DOCUMENT-NUMBER: 20010036625

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010036625 A1

TITLE: Method for identifying compounds for treatment of insulin resistance

PUBLICATION-DATE: November 1, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Shoelson, Steven	Natick	MA	US	

APPL-NO: 09/ 776432

DATE FILED: February 2, 2001

RELATED-US-APPL-DATA:

child 09776432 A1 20010202

parent continuation-in-part-of 09636150 20000810 US PENDING

non-provisional-of-provisional 60148037 19990810 US

US-CL-CURRENT: 435/4, 424/9.2 , 514/12

ABSTRACT:

The invention features a method of identifying, evaluating or making a compound or agent, e.g., a candidate compound or agent, for treatment of a disorder characterized by insulin resistance. The method includes evaluating the ability of a compound or agent to bind IKK-.beta. or modulate IKK-.beta. activity, to thereby identify a compound or agent for the treatment of a disorder characterized by insulin resistance. The invention also features compounds for treating insulin resistance identified by such methods, and methods of treating a subject having a disorder characterized by insulin resistance by administering such agents.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part application of and claims priority to U.S. application Ser. No. 09/636,150, filed on Aug. 10, 2000, and U.S. Provisional Application Serial No. 60/148,037, filed Aug. 10, 1999, the contents of which are incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (14):

[0013] In a preferred embodiment, the ability of a test compound to bind **IKK**-.beta. can be determined by detecting the formation of a **complex** between **IKK**-.beta. and the compound. The presence of the compound in **complex** indicates the ability to bind **IKK**-.beta..

Summary of Invention Paragraph - BSTX (25):

[0024] In a preferred embodiment, the ability of a test compound to bind **IKK**-.beta. can be determined by detecting the formation of a **complex** between **IKK**-.beta. and the compound. The presence of the compound in **complex** indicates the ability to bind **IKK**-.beta..

Detail Description Paragraph - DETX (28):

[0074] Culture cells were used as follows to investigate the mechanisms relating to salicylate treatment to the in vivo reversal of insulin resistance. TNF-.alpha. treatment of 3T3-L1 adipocytes induced `insulin resistance`, as judged by significant decreases in insulin-stimulated tyrosine phosphorylation of IR .beta.-subunit (42.+-.11%) and IRS-1 (37.+-.9%). TNF-.alpha. mediated `insulin resistance` was reversed by pretreatment with high-dose (5 mM) aspirin. IR and IRS-1 phosphorylation levels were restored to 126.+-.24% and 136.+-.35%, respectively, compared to untreated controls; IR and IRS-1 protein levels were unchanged in TNF-.alpha. and aspirin-treated cells. TNF-.alpha. activates a cascade of adapters and kinases, including TRADD, RIP, TRAF2, and TAB1, which act upstream of JNK, p38 MAPK, and the **IKK complex**. Okadaic acid and calyculin A, two phosphatase inhibitors, also activate IKK.beta. (DiDonato et al. (1997) Nature 388:548; Harhaj & Sun (1997) J Biol Chem 272:5409), but without activating upstream elements in the TNF-.alpha. signaling cascade. Okadaic acid and calyculin A also induce `insulin resistance` in isolated tissues and cultured cells (Robinson et al (1993) Am J Physiol 265:E36; Paz et al (1997) J.Biol Chem 272:29911). Therefore, it was determined whether aspirin would reverse `insulin resistance` caused by these inhibitors. Marked reductions in insulin-stimulated IR (29.+-.12%) and IRS-1 (16.+-.2%) tyrosine-phosphorylation were prevented by incubating the cells with high-dose aspirin (109.+-.15% and 93.+-.25%, respectively). Notably, the reduced electrophoretic mobility of IRS-1 due to calyculin A-induced phosphorylation was reversed with aspirin, further suggesting that aspirin's ability to reverse insulin resistance might occur through reduced Ser/Thr phosphorylation of components in the insulin signaling cascade.

Detail Description Paragraph - DETX (32):

[0078] TNF-.alpha. does not appear to contribute to insulin resistance in type 2 diabetes and syndrome X, as biological blockers of TNF-.alpha. do not alter insulin sensitivity (Ofei et al. (1996) Diabetes 45:881; Paquot et al. (2000) J Clin Endocrinol Metab 85:1316). However, TNF-.alpha. is a potential mediator of acquired insulin resistance (Lang et al. (1992) Endocrinology

130:43; Feinstein et al. (1993) J Biol Chem 268:26055; Hotamisligil et al. (1993) Science 259:87; Hotamisligil et al. (1994) J Clin Invest 94:1543). TNF-.alpha. activates the **IKK complex**. TNF-.alpha. treatment of untransfected 293 cells reduced insulin-stimulated IR activation to 29.+-.2% of untreated controls. Expression of kinase deficient, dominant inhibitory IKK.alpha.(K44A) or IKK.beta.(K44A) reversed TNF-.alpha.-inhibited IR activation. In fact, dominant-inhibitory IKK.beta. caused a 60% increase in insulin-stimulated IR tyrosine-phosphorylation over controls, whether or not cells had been treated with TNF-.alpha.. Similar effects were seen with AKT. TNF-.alpha. treatment reduced AKT activation (18.+-.15%), and this was reversed by IKK.beta.(K44A) expression (174.+-.38%). Active **IKK** kinases thus mediate `insulin resistance` in cultured cells, and the inactive kinases act as dominant inhibitors to block TNF-.alpha. induced insulin resistance. The consistent ability of dominant-inhibitory IKK.beta. to elevate IR signaling well above the normal level indicates that **IKK** inhibits insulin signaling even in the absence of TNF-.alpha.. There is in vivo support for this, as well. Fa/+ rats and ob/+ mice (see FIG. 1) and Sprague-Dawley rats that are not insulin resistant, obese, or diabetic, show increased insulin sensitivity in response to aspirin treatment.

Claims Text - CLTX (8):

7. The method of claim 2, wherein the ability of the compound to bind **IKK**-.beta. is determined by detecting the formation of a **complex** between **IKK**-.beta. and the compound.

US-PAT-NO: 6489151

DOCUMENT-IDENTIFIER: US 6489151 B1

TITLE: Biologically active alternative form of the IKK.alpha. I.kappa.B kinase

DATE-ISSUED: December 3, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Marcu; Kenneth B.	Stony Brook	NY	N/A	N/A
Connelly; Margery A.	Medford	NY	N/A	N/A

APPL-NO: 09/ 536882

DATE FILED: March 27, 2000

US-CL-CURRENT: 435/194, 536/23.2

ABSTRACT:

The present invention provides isolated I.kappa.B kinases that regulate NF.kappa.B gene transcription that lack both a leucine zipper like .alpha.-helix domain and helix-loop-helix domain. Also provided are the amino acid sequences of these kinases and the nucleotide sequence encoding these kinases, and other related protein and nucleic acid molecules.

16 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

---------- KWIC ---------

Detailed Description Text - DETX (6):

Akin to IKK.alpha./CHUK, the IKK.alpha.-.DELTA.LH and IKK.alpha.-.DELTA.Cm proteins are TNF-.alpha. inducible, NF-.kappa.B activating I.kappa.B.alpha. kinases. By a combination of NF-.kappa.B element driven luciferase gene reporter assays, immune **complex** kinase assays and co-immunoprecipitations with other known components of the approximately 700-900 kD **IKK complex,** the IKK.alpha.-.DELTA.LH and IKK.alpha.-.DELTA.Cm proteins were found to behave in a similar fashion to full length IKK.alpha./CHUK by several criteria. First, expression plasmid dose response curves reveal that each form of IKK.alpha./CHUK activates a comparable level of NF-.kappa.B luciferase activity

even at their limiting dosages (FIG. 4B). Second, each form of IKK.alpha./CHUK correctly phosphorylates I.kappa.B.alpha. (on serines 32 and 36) in response to TNF.alpha. signaling (FIG. 5A). Third, IKK.alpha.-.DELTA.Cm activates NF-.kappa.B and phosphorylates I.kappa.B.alpha. with an enzymatic time course superimposable with full length IKK.alpha./CHUK. (FIG. 5B.) Fourth, like IKK.alpha./CHUK, IKK.alpha.-.DELTA.Cm's ability to activate NF-.kappa.B is not appreciably enhanced by co-expression with IKK.beta. and is inhibited by a kinase inactive, ATP binding domain mutant of IKK.alpha./CHUK. Therefore, these isoforms of IKK.alpha./CHUK, which lack the LZ and H-L-H domains, retain a number of functions of the full length IKK.alpha./CHUK. It is surprising that the carboxy-tail domain of the full length IKK.alpha./CHUK does not significantly contribute to the kinase's functional activity.

Other Reference Publication - OREF (9):

Zandi et al., "The I.kappa.B Kinase **Complex (IKK**) Contains Two Kinase Subunits, IKK.alpha. and IKK.beta., Necessary for I.kappa.B Phosphorylation and NF-.kappa.B Activation", Cell vol. 91: 243-252 (1997).

US-PAT-NO: 6479266

DOCUMENT-IDENTIFIER: US 6479266 B1

TITLE: IKK-.alpha. proteins nucleic acids and methods

DATE-ISSUED: November 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rothe; Mike	San Mateo	CA	N/A	N/A
Cao; Zhaodan	Pacifica	CA	N/A	N/A
Regnier; Catherine	South San Francisco	CA	N/A	N/A

APPL-NO: 09/ 109986

DATE FILED: July 2, 1998

PARENT-CASE:

This is a continuing application of U.S. Ser. No. 08/890,854, filed Jul. 10, 1997, which is a continuing application under 35USC120 of U.S. Ser. No. 08/887,115, now abandoned, filed Jul. 1, 1997.

US-CL-CURRENT: 435/194, 435/15

ABSTRACT:

The invention provides methods and compositions relating to an I.kappa.B kinase, IKK-.alpha., and related nucleic acids. The polypeptides may be produced recombinantly from transformed host cells from the disclosed IKK-.alpha. encoding nucleic acids or purified from human cells. The invention provides isolated IKK-.alpha. hybridization probes and primers capable of specifically hybridizing with the disclosed IKK-.alpha. genes, IKK-.alpha.-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

20 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (7):

Here, we disclose a novel human kinase I.kappa.B Kinase, **IKK**-.alpha., as a NIK-interacting protein. **IKK**-.alpha. has sequence similarity to the

conceptual translate of a previously identified open reading frame (SEQ ID NO:5) postulated to encode a serine-threonine kinase of unknown function (`Conserved Helix-loop-helix Ubiquitous Kinase` or CHUK, Connelly and Marcu, 1995; Mock et al., 1995). Catalytically inactive mutants of **IKK**-.alpha. are shown to suppress NF-.kappa.B activation induced by TNF and IL-1 stimulation as well as by TRAF and NIK overexpression; transiently expressed **IKK**-.alpha. is shown to associate with the endogenous I.kappa.B.alpha. **complex; and** **IKK**-.alpha. is shown to phosphorylate I.kappa.B.alpha. on serines 32 and 36.

Brief Summary Text - BSTX (17):

The claimed **IKK**-.alpha. polypeptides are isolated or pure: an "isolated" polypeptide is unaccompanied by at least some of the material with which it is associated in its natural state, preferably constituting at least about 0.5%, and more preferably at least about 5% by weight of the total polypeptide in a given sample and a pure polypeptide constitutes at least about 90%, and preferably at least about 99% by weight of the total polypeptide in a given sample. In a particular embodiments, **IKK**-.alpha. polypeptides are isolated from a MKP-1 precipitable **complex,** isolated from a **IKK complex,** and/or isolated from **IKK**-.beta.. The **IKK**-.alpha. polypeptides and polypeptide domains may be synthesized, produced by recombinant technology, or purified from mammalian, preferably human cells. A wide variety of molecular and biochemical methods are available for biochemical synthesis, molecular expression and purification of the subject compositions, see e.g. Molecular Cloning, A Laboratory Manual (Sambrook, et al. Cold Spring Harbor Laboratory), Current Protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY) or that are otherwise known in the art.

Detailed Description Text - DETX (6):

The interaction of **IKK**-.alpha. with NIK was confirmed in mammalian cell coimmunoprecipitation assays. Human **IKK**-.alpha. containing an N-terminal Flag epitope tag was transiently coexpressed in 293 human embryonic kidney cells with Myc epitope-tagged NIK or HA epitope-tagged TRAF proteins. Cell lysates were immunoprecipitated using a monoclonal antibody against the Flag epitope, and coprecipitating NIK or TRAF proteins were detected by immunoblot analysis with an anti-Myc or anti-HA monoclonal antibodies. In this assay, **IKK**-.alpha. was able to coprecipitate NIK confirming the interaction between both proteins as detected for **IKK**-.alpha. by yeast two-hybrid analysis. Also, a deletion mutant **IKK**-.alpha. protein lacking most of the N-terminal kinase domain (**IKK**-.alpha..sub.(307-745)) was able to associate with NIK, indicating that the .alpha.-helical C-terminal half of **IKK**-.alpha. mediates the interaction with NIK. In contrast to NIK **IKK**-.alpha. failed to associate with either TRAF2 or TRAF3. However, when NIK was coexpressed with **IKK**-.alpha. and TRAF2, strong coprecipitation of TRAF2 with **IKK**-.alpha. was detected, indicating the formation of a ternary **complex** between **IKK**-.alpha., NIK and TRAF2.

US-PAT-NO: 6468755

DOCUMENT-IDENTIFIER: US 6468755 B1

TITLE: Method for identifying compounds for treatment of insulin resistance

DATE-ISSUED: October 22, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Shoelson; Steven	Natick	MA	N/A	N/A

APPL-NO: 09/ 636150

DATE FILED: August 10, 2000

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Serial No. 60/148,037, filed on Aug. 10, 1999, which is incorporated herein by reference in its entirety.

US-CL-CURRENT: 435/7.1, 435/174 , 435/7.8 , 514/2

ABSTRACT:

The invention features a method of identifying, evaluating or making a compound or agent, e.g., a candidate compound or agent, for treatment of a disorder characterized by insulin resistance. The method includes evaluating the ability of a compound or agent to interact with, e.g., bind, IKK-.beta., to thereby identify a compound or agent for the treatment of a disorder characterized by insulin resistance. The invention also features compounds for treating insulin resistance identified by such methods, and methods of treating a subject having a disorder characterized by insulin resistance by administering such agents.

12 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (10):

In a preferred embodiment, the ability of a test compound to bind **IKK**-.beta.

can be determined by detecting the formation of a **complex** between **IKK**-.beta. and the compound. The presence of the compound in **complex** indicates the ability to bind **IKK**-.beta..

Brief Summary Text - BSTX (21):

In a preferred embodiment, the ability of a test compound to bind **IKK**-.beta. can be determined by detecting the formation of a **complex** between **IKK**-.beta. and the compound. The presence of the compound in **complex** indicates the ability to bind **IKK**-.beta..

Claims Text - CLTX (3):

3. The method of claim 2, wherein determining whether the test compound inhibits **IKK**-.beta. activity comprises detecting the formation of a **complex** between **IKK**-.beta. and the compound.

Other Reference Publication - OREF (4):

Rothwarf et al., "**IKK**-.gamma. is an essential regulatory subunit of the IkB kinase **complex**", Nature, 395-297-300, Sep., 1998.

US-PAT-NO: 6428950

DOCUMENT-IDENTIFIER: US 6428950 B1

TITLE: Assay to identify compounds that alter apolipoprotein E expression

DATE-ISSUED: August 6, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cordell; Barbara	Palo Alto	CA	N/A	N/A
Xu; Qiang	Cupertino	CA	N/A	N/A
Naidu; Asha	Fremont	CA	N/A	N/A
Paul; Steven M.	Carmel	IN	N/A	N/A
Bales; Kelly R.	Cloverdale	IN	N/A	N/A

APPL-NO: 09/ 447452

DATE FILED: November 22, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. .sctn.119(e) to U.S. Provisional Application No. 60/109,910, filed Nov. 25, 1998.

US-CL-CURRENT: 435/4, 424/570 , 424/572 , 424/577 , 435/7.21 , 435/70.3 , 514/1

ABSTRACT:

The present invention provides a method of assaying for and arresting, preventing and/or reversing the impairment of central and peripheral nervous system function comprising reducing .beta.-amyloid plaque burden by the administration of compounds that reduce apoE expression. The compounds used in the method of the invention may be: 1) inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase; 2) inhibitors of cholesterol biosynthesis; 3) inhibitors of protein isoprenylation, specifically geranylgeranylation; and/or 4) inhibitors of NF-.kappa.B activation or function. Assays for compounds with inhibit apoE expression from microglial cells are also disclosed.

2 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

---------- KWIC ---------

Detailed Description Text - DETX (39):

Important modulators of NF-.kappa.B activation are the inhibitor proteins I.kappa.B.sub..alpha. and I.kappa.B.sub..beta. (referred to herein as I.kappa.B), which associate with, and thereby, inactivate, NF-.kappa.B in vivo. Activation and nuclear translocation of NF-.kappa.B occurs following signal-induced phosphorylation of I.kappa.B, which leads to proteolysis via the ubiquitin pathway. This pathway includes a cascade of atypical protein kinases that catalyze the ubiquination of I.kappa.B, the I.kappa.B kinase (**IKK) complex** (FIG. 4).

US-PAT-NO: 6395545

DOCUMENT-IDENTIFIER: US 6395545 B1

TITLE: Antisense modulation of inhibitor-kappa B kinase-alpha expression

DATE-ISSUED: May 28, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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APPL-NO: 09/ 856074

DATE FILED: July 27, 2001

PARENT-CASE:

INTRODUCTION

This application is the U.S. National Phase of PCT/US99/16603 filed Jul. 22, 1999, which is a continuation of U.S. patent application Ser. No. 09/197,360, filed Nov. 20, 1998, now issued as U.S. Pat. No. 5,962,673.

PCT-DATA:
APPL-NO: PCT/US99/16603
DATE-FILED: July 22, 1999
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PUB-DATE: Jun 2, 2000
371-DATE: Jul 27, 2001
102(E)-DATE:Jul 27, 2001

US-CL-CURRENT: 435/375, 435/455 , 435/6 , 536/23.1 , 536/24.3 , 536/24.31 , 536/24.33 , 536/24.5

ABSTRACT:

Antisense compounds, compositions and methods are provided for modulating the expression of Inhibitor-kappa B Kinase-alpha. The compositions comprise antisense compounds, particularly antisense oligonucleotides, targeted to nucleic acids encoding Inhibitor-kappa B Kinase-alpha. Methods of using these compounds for modulation of Inhibitor-kappa B Kinase-alpha expression and for treatment of diseases associated with expression of Inhibitor-kappa B Kinase-alpha are provided.

15 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Other Reference Publication - OREF (8):

Zandi et al., "The IkappaB kinase **complex (IKK**) contains two kinase subunits, IKKalpha and IKKbeta, necessary for IkappaB phosphorylation and NF-kappaB activation", Cell 1997 91:243-252.

US-PAT-NO: 6365722

DOCUMENT-IDENTIFIER: US 6365722 B1
See image for Certificate of Correction

TITLE: Y2H14 an IKK binding protein

DATE-ISSUED: April 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Marcu; Kenneth B.	Stony Brook	NY	N/A	N/A

APPL-NO: 09/ 195188

DATE FILED: November 17, 1998

US-CL-CURRENT: 536/23.1, 530/350

ABSTRACT:

The present invention provides an isolated I.kappa.B kinase binding protein designated Y2H14 and functional equivalents therof. The amino acid sequence of Y2H14, the nucleotide sequence encoding Y2H14, and other related protein and nucleic acid molecules are also provided.

6 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Other Reference Publication - OREF (8):

Zandi et al., "The I.kappa.B Kinase **Complex (IKK**) Contains Two Kinase Subunits, IKK.alpha. and IKK.beta., Necessary for I.kappa.B Phosphorylation and NF-.kappa.B Activation", Cell 91: 243-252 (1997).

US-PAT-NO: 6365366

DOCUMENT-IDENTIFIER: US 6365366 B1

TITLE: T2k kinase assays

DATE-ISSUED: April 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cao; Zhaodan	South San Francisco	CA	N/A	N/A

APPL-NO: 09/ 524435

DATE FILED: March 13, 2000

US-CL-CURRENT: 435/21, 435/15 , 435/4 , 435/7.1 , 435/968 , 530/300

ABSTRACT:

T2K kinase activity is detected by forming a mixture of a T2K kinase and a substrate; incubating the mixture under conditions whereby the kinase phosphorylates the substrate at a first rate; and detecting the first rate as an indication of the kinase activity. The substrate comprises SX.sub.1 X.sub.2 X.sub.3 SX.sub.4 (SEQ ID NO:1) wherein X.sub.1 and X.sub.4 are aliphatic residues and both of the S residues are targets of the kinase, and especially, IKK.alpha. or IKK.beta.. In another embodiment, the mixture substrate comprises a particular IL-1 or TNF signaling cascade component. The mixture may be used to screen for agents which modulate the activity of the kinase, e.g. as an immuno-chemiluminescent assay.

17 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (4):

Inflammatory cytokines IL-1 and TNF exert diverse biological activities by altering gene expression in the cells, a function mediated in part by transcription factor NF-.kappa.B. In unstimulated cells, NF-.kappa.B proteins form a **complex** with inhibitory molecules, the I.kappa.B proteins, and are rendered inactive in the cytoplasm. In response to cytokines and other stimuli, the I.kappa.B proteins are phosphorylated on specific serine residues. Delineating TNF and IL-1 signaling pathways for NF-.kappa.B activation has implicated the TRAF molecules as converging point for different cytokines, with TRAF2 being involved in TNF- and TRAF6 in IL-1-induced NF-.kappa.B activation.

We previously disclosed a family of I.kappa.B kinases including a TRAF2-associated kinase activity (designated T2K) and the translation product of the KIAA0151 gene product (also known as **IKKi** and IKK.epsilon.) that phosphorylates the I.kappa.B molecules on the specific regulatory serine residues. We have now found that T2K and **IKKi** in fact have alternative substrate specificities and alternative physiologically relevant substrate targets, particularly IKK.alpha. and IKK.beta. peptide substrates. We disclose here materials and methods for assaying for these novel specificities.

US-PAT-NO: 6265538

DOCUMENT-IDENTIFIER: US 6265538 B1

TITLE: Inhibitor of the inflammatory response induced by the TNFA and IL-1

DATE-ISSUED: July 24, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Greene; Warner C.	Hillsborough	CA	N/A	N/A
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APPL-NO: 09/ 257703

DATE FILED: February 25, 1999

PARENT-CASE:

This application claims priority of U.S. Ser. No. 60/076,299, filed Feb. 27, 1998.

US-CL-CURRENT: 530/324, 435/7.1 , 530/350

ABSTRACT:

The present invention provides the molecular basis for cytokine induction of NF-.kappa.B-dependent immune and inflammatory responses, emphasizing a role for both NIK-NIK and NIK-IKK protein-protein interactions. A relatively small region of NIK selectively impairs the NIK-IKK interaction. The present invention provides a highly specific method for modulating NF-.kappa.B-dependent immune, inflammatory, and anti-apoptotic responses, based on interruption of the critical protein-protein interaction of NIK and IKK. The present invention provides methods for inhibiting NF-.kappa.B-dependent gene expression, using mutant NIK proteins. One embodiment of the present invention provides kinase-deficient NIK mutant proteins that inhibit activation of IKK. Another embodiment of the invention provides N-terminus NIK mutant proteins that bind IKK, thus inhibiting NIK/IKK interaction.

2 Claims, 25 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

---------- KWIC ---------

Detailed Description Text - DETX (9):

The term "NIK/**IKK**" interaction used herein is defined as NIK/**IKK** protein binding, a NIK/**IKK** protein **complex** and NIK/**IKK** protein assembly.

Detailed Description Text - DETX (10):

The term "inhibition of NIK/**IKK** interaction" used herein is defined as inhibiting either the formation of a NIK/**IKK** protein **complex** or disruption of a formed NIK/**IKK** protein **complex**.

Detailed Description Text - DETX (12):

The term "NF-.kappa.B-dependent gene expression" used herein is defined as those immune and inflammatory genes that are under the regulatory control of the .kappa.B-enhancer. In most cells, NF-.kappa.B exists in a latent state in the cytoplasm bound to inhibitory proteins, collectively called I.kappa.B, that mask the nuclear localization signal thereby preventing nuclear translocation. The latent form of NF-.kappa.B can be induced by cytokines, such as TNF.alpha. and IL-1. Both TNF.alpha. and IL-1 signaling leads to sequential phosphorylation and activation of a series of proteins involved in a cascade pathway that requires NIK/**IKK** protein interaction and **IKK** activation, that in turn leads to phosphorylation and degradation of I.kappa.B. Degradation of the I.kappa.B inhibitor unmasks the nuclear localization signal of the NF-.kappa.B **complex** allowing its rapid translocation into the nucleus where it engages cognate .kappa.B-enhancer elements and activates the transcription of various NF-.kappa.B-dependent genes involved in inflammation and immune response.

Detailed Description Text - DETX (21):

One embodiment of the invention provides a method for inhibiting activation of NF-.kappa.B-dependent gene expression by inhibiting NIK/**IKK** interaction, by contacting an **IKK** protein with a catalytically inactive NIK protein that continues to interact with and bind to **IKK**. The inhibition of the NIK/**IKK** interaction includes inhibiting the formation of a NIK/**IKK complex**, or disruption of a formed NIK/**IKK complex**. One particular embodiment of the present invention provides a method for inhibiting activation of NF-.kappa.B-dependent gene expression by inhibiting wild type NIK/**IKK** interaction, by contacting the wild type **IKK**/NIK protein **complex** with a catalytically inactive or mutant NIK protein, such that the mutant NIK competes with the wild type NIK for assembly with the **IKK**, thereby forming an inactive NIK/**IKK** protein **complex**. One embodiment provides using an N-terminus deletion mutant NIK protein, such as N735-947, to inhibit NIK/**IKK** interaction. These N-terminus mutant NIK proteins bind to **IKK** protein and inhibit wild type NIK/**IKK** interaction, thereby inhibiting activation of NF-.kappa.B-dependent gene expression. The methods of the present invention are not limited to using only the N-terminus deletion mutant NIK protein N735-947, as the present invention also provides for use of other N-terminus deletion mutant NIK

proteins and other types of NIK mutant proteins that can interact with an **IKK** protein to inhibit activation of NF-.kappa.B-dependent gene expression.

Detailed Description Text - DETX (115):

Zandi, E., D. M. Rothwarf, M. Delhase, M. Hayakawa and M. Karin. 1997. The IkappaB kinase **complex (IKK)** contains two kinase subunits, IKKalpha and IKKbeta, necessary for IkappaB phosphorylation and NF-kappaB activation. Cell 91:243-52

Other Reference Publication - OREF (1):

Zandi, Ebrahim et al., "T.kappa.B Kinase **Complex (IKK)** Contains Two Kinase Subunits, IKK.alpha. and IKK.beta., Necessary for I.kappa.B Phosphorylation and NF-.kappa.B Activation," Cell, Oct. 17, 1997, 91:243-52. (Exhibit 30).

US-PAT-NO: 6262228

DOCUMENT-IDENTIFIER: US 6262228 B1

TITLE: IRAK3 polypeptides and methods

DATE-ISSUED: July 17, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cao; Zhaodan	South San Francisco	CA	N/A	N/A

APPL-NO: 09/ 135232

DATE FILED: August 17, 1998

US-CL-CURRENT: 530/300, 435/7.1 , 435/7.2 , 530/324 , 530/326 , 530/327 , 530/328 , 530/329 , 530/350 , 530/351

ABSTRACT:

The invention provides methods and compositions relating to a novel kinase, IRAK3. The polypeptides may be produced recombinantly from transformed host cells from the disclosed IRAK3 encoding nucleic acids or purified from human cells. The invention provides isolated IRAK3 hybridization probes and primers capable of specifically hybridizing with the disclosed IRAK3 genes, IRAK3-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

38 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (4):

Interleukin 1 (IL-1) receptor associated kinase (IRAK) functions as an intracellular signal transducer for the pro-inflammatory cytokine IL-1. IL-1 treatment of cells induces the **complex** formation of the two IL-1 receptor chains, IL-1R1 and IL-1RAcP, which recruits an adaptor molecule designated as MyD88 which binds to IRAK. IRAK is subsequently phosphorylated, released from the receptor **complex** to interact with TRAF6. TRAF6 triggers either the NIK/**IKK** kinase cascade to activate the transcription factor NF-.kappa.B or an undefined kinase cascade to activate the transcription factor AP-1. Both transcription factors regulate large numbers of genes that regulate immune and inflammatory responses.

US-PAT-NO: 6258579

DOCUMENT-IDENTIFIER: US 6258579 B1

TITLE: Stimulus-inducible protein kinase complex and methods of use therefor

DATE-ISSUED: July 10, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mercurio; Frank	San Diego	CA	N/A	N/A
Zhu; Hengyi	San Diego	CA	N/A	N/A
Barbosa; Miguel	San Diego	CA	N/A	N/A
Li; Jian Wu	San Diego	CA	N/A	N/A
Murray; Brion W.	San Diego	CA	N/A	N/A

APPL-NO: 08/ 910820

DATE FILED: August 13, 1997

PARENT-CASE:

CROSS-REFERENCE TO PRIOR APPLICATION

This application is a continuation-in-part of U.S. patent application Ser. No. 08/697,393, filed Aug. 26, 1996 now U.S. Pat. No. 5,972,674.

US-CL-CURRENT: 435/194

ABSTRACT:

Compositions and methods are provided for treating NF-.kappa.B-related conditions. In particular, the invention provides a stimulus-inducible IKK signalsome, and components and variants thereof. An IKK signalsome or component thereof may be used, for example, to identify antibodies and other modulating agents that inhibit or activate signal transduction via the NF-.kappa.B cascade. IKK signalsome, components thereof and/or modulating agents may also be used for the treatment of diseases associated with NF-.kappa.B activation.

4 Claims, 30 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 28

---------- KWIC ---------

Drawing Description Text - DRTX (12):

FIGS. 8A-8C are autoradiograms depicting the results of immunoblot analyses. In FIG. 8A, the upper panel presents a time course for the induction of signalsome activity. Anti MKP-1 immune precipitates from extracts of HeLa S3 cells stimulated with TNF.alpha. (20 ng/ml) for the indicated times, were assayed for **IKK** signalsome activity by standard immune **complex** kinase assays. 4 .mu.g of either GST I.kappa.B.alpha. 1-54 WT (wildtype) or the GST I.kappa.B.alpha. 1-54 S32/36 to T mutant (S>T) were used as the substrates. In the lower panel, HeLa cell extracts prepared as described in the upper panel were examined by western blot analysis for I.kappa.B.alpha. degradation. I.kappa.B.alpha. supershifting phosphorylation can be seen after 3 and 5 minutes of stimulation followed by the disappearance of I.kappa.B.alpha..

Drawing Description Text - DRTX (14):

FIG. 8C illustrates the ability of **IKK** signalsome to specifically phosphorylate serines 32 and 36 of the I.kappa.B.alpha. holoprotein in the context of a RelA:I.kappa.B.alpha. **complex**. Anti-MKP-1 immunoprecipitates from cell extracts of HeLa S3 cells stimulated with TNF.alpha. (20 ng/ml, 7 min) were examined for their ability to phosphorylate baculoviral expressed RelA:I.kappa.B.alpha. **complex** containing either the I.kappa.B.alpha. WT (lane 3) or I.kappa.B.alpha. S32/36 to A mutant (lane 4) holoprotein. The specific substrates used are indicated on the top. Positions of the phosphorylated substrates are indicated by arrows to the left of the panel.

Drawing Description Text - DRTX (17):

FIG. 10 is an autoradiogram showing the results of a western blot analysis of the level of ubiquitin within a stimulus-inducible IkB kinase **complex**. Lane I shows the detection of 100 ng ubiquitin, Lane 2 shows 10 ng ubiquitin and Lane 3 shows 3.4 .mu.g of **IKK** signalsome purified through the phenyl superose step (sufficient quantities for 10 kinase reactions). The position of ubiquitin is shown by the arrow on the left.

Detailed Description Text - DETX (2):

As noted above, the present invention is generally directed to compositions and methods for modulating (i.e., stimulating or inhibiting) signal transduction leading to NF-.kappa.B activation. In particular, the present invention is directed to compositions comprising an I.kappa.B kinase (**IKK**) signalsome (also referred to herein as a "stimulus-inducible I.kappa.B kinase **complex**" or "I.kappa.B kinase **complex**") that is capable of stimulus-dependent phosphorylation of I.kappa.B.alpha. and I.kappa.B.beta. on the two N-terminal serine residues critical for the subsequent ubiquitination and degradation in vivo. Such stimulus-dependent phosphorylation may be achieved without the addition of exogenous cofactors. In particular, an **IKK** signalsome specifically phosphorylates I.kappa.B.alpha. (SEQ ID NO:1) at residues S32 and S36 and

phosphorylates I.kappa.B.beta. (SEQ ID NO:2) at residues S19 and S23. The present invention also encompasses compositions that contain one or more components of such an **IKK** signalsome, or variants of such components. Preferred components, referred to herein as "**IKK** signalsome kinases" "I.kappa.B kinases" or **IKKs**) are kinases that, when incorporated into an **IKK** signalsome, are capable of phosphorylating I.kappa.B.alpha. at S32 and S36. Particularly preferred components are **IKK-1** (SEQ ID NO:10) and **IKK-2** (SEQ ID NO:9).

Detailed Description Text - DETX (4):

An **IKK** signalsome has several distinctive properties. Such a **complex** is stable (i.e., its components remain associated following purification as described herein) and has a high-molecular weight (about 500-700 kD, as determined by gel filtration chromatography). As shown in FIGS. 3(A and B) and 4(A and B), I.kappa.B kinase activity of an **IKK** signalsome is "stimulus-inducible" in that it is stimulated by TNF.alpha. (i.e., treatment of cells with TNF.alpha. results in increased I.kappa.B kinase activity and I.kappa.B degradation) and/or by one or more other inducers of NF-.kappa.B, such as IL-1, LPS, TPA, UV irradiation, antigens, viral proteins and stress-inducing agents. The kinetics of stimulation by TNF.alpha. correspond to those found in vivo. I.kappa.B kinase activity of an **IKK** signalsome is also specific for S32 and S36 of I.kappa.B.alpha.. As shown in FIGS. 5(A and B) and 6(A and B), an **IKK** signalsome is capable of phosphorylating a polypeptide having the N-terminal sequence of I.kappa.B.alpha. (GST-I.kappa.B.alpha.1-54; SEQ ID NO:3), but such phosphorylation cannot be detected in an I.kappa.B.alpha. derivative containing threonine substitutions at positions 32 and 36. In addition, I.kappa.B kinase activity is strongly inhibited by a doubly phosphorylated I.kappa.B.alpha. peptide (i.e., phosphorylated at S32 and S36), but not by an unrelated c-fos phosphopeptide that contains a single phosphothreonine. A further characteristic of an **IKK** signalsome is its ability to phosphorylate a substrate in vitro in a standard kinase buffer, without the addition of exogenous cofactors. Free ubiquitin is not detectable in preparations of **IKK** signalsome (see FIG. 10), even at very long exposures. Accordingly an **IKK** signalsome differs from the ubiquitin-dependent I.kappa.B.alpha. kinase activity described by Chen et al., Cell 84:853-62, 1996.

Detailed Description Text - DETX (5):

An **IKK** signalsome may be immunoprecipitated by antibodies raised against MKP-1 (MAP kinase phosphatase-1; Santa Cruz Biotechnology, Inc., Santa Cruz, Calif. #SC-1102), and its activity detected using an in vitro I.kappa.B.alpha. kinase assay. However, as discussed further below, MKP-1 does not appear to be a component of I.kappa.B kinase **complex**. The substrate specificity of the immunoprecipitated **IKK** signalsome is maintained (i.e., there is strong phosphorylation of wildtype GST-I.kappa.B.alpha. 1-54 (SEQ ID NO:3) and GST-I.kappa.B.beta. 1-44 (SEQ ID NO:4), and substantially no detectable phosphorylation of GST-I.kappa.B.alpha.. 1-54 in which serines 32 and 36 are replaced by threonines (GST- I.kappa.B.alpha. S32/36 to T; SEQ ID NO:5) or GST-I.kappa.B.beta. 1-44 in which serines 19 and 23 are replaced by alanines

(GST-I.kappa.B.beta. 1-44 S19/23 to A; SEQ ID NO:6)).

Detailed Description Text - DETX (8):

Throughout the fractionation, an in vitro kinase assay may be used to monitor the I.kappa.B kinase activity of the **IKK** signalsome. In such an assay, the ability of a fraction to phosphorylate an appropriate substrate (such as I.kappa.B.alpha. (SEQ ID NO:1) or a derivative or variant thereof) is evaluated by any of a variety of means that will be apparent to those of ordinary skill in the art. For example, a substrate may be combined with a chromatographic fraction in a protein kinase buffer containing .sup.32 P .gamma.-ATP, phosphatase inhibitors and protease inhibitors. The mixture may be incubated for 30 minutes at 30.degree. C. The reaction may then be stopped by the addition of SDS sample buffer and analyzed using SDS-PAGE with subsequent autoradiography. Suitable substrates include full length I.kappa.B.alpha. (SEQ ID NO: 1), polypeptides comprising the N-terminal 54 amino acids of I.kappa.B.alpha., full length I.kappa.B.beta. (SEQ ID NO:2) and polypeptides comprising the N-terminal 44 amino acids of I.kappa.B.beta.. Any of these substrates may be used with or without an N-terminal tag. One suitable substrate is a protein containing residues 1-54 of I.kappa.B.alpha. and an N-terminal GST tag (referred to herein as GST-I.kappa.B.alpha. 1-54; SEQ ID NO:3). To evaluate the specificity of an I.kappa.B kinase **complex,** I.kappa.B.alpha. mutants containing threonine or alanine residues at positions 32 and 36, and/or other modifications, may be employed.

Detailed Description Text - DETX (9):

Alternatively, an **IKK** signalsome may be prepared from its components which are also encompassed by the present invention. Such components may be produced using well known recombinant techniques, as described in greater detail below. Components of an **IKK** signalsome may be native, or may be variants of a native component (i.e., a component sequence may differ from the native sequence in one or more substitutions and/or modifications, provided that the ability of a **complex** comprising the component variant to specifically phosphorylate I.kappa.B.alpha. is not substantially diminished). Substitutions and/or modifications may generally be made in non-critical and/or critical regions of the native protein. Variants may generally comprise residues of L-amino acids, D-amino acids, or any combination thereof. Amino acids may be naturally-occurring or may be non-natural, provided that at least one amino group and at least one carboxyl group are present in the molecule; .alpha.- and .beta.-amino acids are generally preferred. A variant may also contain one or more rare amino acids (such as 4-hydroxyproline or hydroxylysine), organic acids or amides and/or derivatives of common amino acids, such as amino acids having the C-terminal carboxylate esterified (e.g., benzyl, methyl or ethyl ester) or amidated and/or having modifications of the N-terminal amino group (e.g., acetylation or alkoxycarbonylation), with or without any of a wide variety of side-chain modifications and/or substitutions (e.g., methylation, benzylation, t-butylation, tosylation, alkoxycarbonylation, and the like). Component variants may also, or alternatively, contain other modifications, including the deletion or addition of amino acids that have minimal influence on the activity of the polypeptide. In particular, variants may contain

additional amino acid sequences at the amino and/or carboxy termini. Such sequences may be used, for example, to facilitate purification or detection of the component polypeptide. In general, the effect of one or more substitutions and/or modifications may be evaluated using the representative assays provided herein.

Detailed Description Text - DETX (11):

Alternatively, partial sequences of the components may be obtained using standard biochemical purification and microsequencing techniques. For example, purified **complex** as described above may be run on an SDS-PAGE gel and individual bands may be isolated and subjected to protein microsequencing. DNA sequences encoding components may then be prepared by amplification from a suitable human cDNA library, using polymerase chain reaction (PCR) and methods well known to those of ordinary skill in the art. For example, an adapter-ligated cDNA library prepared from a cell line or tissue that expresses **IKK** signalsome (such as HeLa or Jurkat cells) may be screened using a degenerate 5' specific forward primer and an adapter-specific primer. Degenerate oligonucleotides may also be used to screen a cDNA library, using methods well known to those of ordinary skill in the art. In addition, known proteins may be identified via Western blot analysis using specific antibodies.

Detailed Description Text - DETX (13):

Particularly preferred components of **IKK** signalsome are I.kappa.B kinases. An I.kappa.B kinase may be identified based upon its ability to phosphorylate one or more I.kappa.B proteins, which may be readily determined using the representative kinase assays described herein. In general, an I.kappa.B kinase is incorporated into an **IKK** signalsome, as described herein, prior to performing such assays, since an I.kappa.B kinase that is not **complex**-associated may not display the same phosphorylation activity as **complex**-associated I.kappa.B kinase. As noted above, an I.kappa.B kinase within an **IKK** signalsome specifically phosphorylates I.kappa.B.alpha. at residues S32 and S36, and phosphorylates I.kappa.B.beta. at residues 19 and 23, in response to specific stimuli.

Detailed Description Text - DETX (22):

In one aspect of the present invention, an **IKK** signalsome and/or one or more components thereof may be used to identify modulating agents, which may be antibodies (e.g., monoclonal), polynucleotides or other drugs, that inhibit or stimulate signal transduction via the NF-.kappa.B cascade. Modulation includes the suppression or enhancement of NF-.kappa.B activity. Modulation may also include suppression or enhancement of I.kappa.B phosphorylation or the stimulation or inhibition of the ability of activated (i.e., phosphorylated) **IKK** signalsome to phosphorylate a substrate. Compositions that inhibit NF-.kappa.B activity by inhibiting I.kappa.B phosphorylation may include one or more agents that inhibit or block I.kappa.B.alpha. kinase activity, such as an antibody that neutralizes **IKK** signalsome, a competing peptide that represents

the substrate binding domain of I.kappa.B kinase or a phosphorylation motif of I.kappa.B, an antisense polynucleotide or ribozyme that interferes with transcription and/or translation of I.kappa.B kinase, a molecule that inactivates **IKK** signalsome by binding to the **complex,** a molecule that binds to I.kappa.B and prevents phosphorylation by **IKK** signalsome or a molecule that prevents transfer of phosphate groups from the kinase to the substrate. Within certain embodiments, a modulating agent inhibits or enhances the expression or activity of **IKK-1** and/or **IKK-2**.

Detailed Description Text - DETX (25):

In another aspect of the present invention, **IKK** signalsome or I.kappa.B kinase may be used for phosphorylating an I.kappa.B such as I.kappa.B.alpha. (or a derivative or variant thereof) so as to render it a target for ubiquitination and subsequent degradation. I.kappa.B may be phosphorylated in vitro by incubating **IKK** signalsome or I.kappa.B kinase with I.kappa.B in a suitable buffer for 30 minutes at 30.degree. C. In general, about 0.01 .mu.g to about 9 .mu.g of I.kappa.B kinase **complex** is sufficient to phosphorylate from about 0.5 .mu.g to about 2 .mu.g of I.kappa.B. Phosphorylated substrate may then be purified by binding to GSH-sepharose and washing. The extent of substrate phosphorylation may generally be monitored by adding [.gamma.-.sup.32 P]ATP to a test aliquot, and evaluating the level of substrate phosphorylation as described herein.

Detailed Description Text - DETX (34):

In another aspect, the present invention provides methods for detecting the level of stimulus-inducible I.kappa.B kinase activity in a sample. The level of I.kappa.B kinase activity may generally be determined via an immunokinase assay, in which **IKK** signalsome is first immunoprecipitated with an antibody that binds to the **complex**. The immunoprecipitated material is then subjected to a kinase assay as described herein. Substrate specificity may be further evaluated as described herein to distinguish the activity of a stimulus-inducible I.kappa.B kinase **complex** from other kinase activities.

Detailed Description Text - DETX (39):

Monoclonal antibodies specific for an **IKK** signalsome or a component thereof may be prepared, for example, using the technique of Kohler and Milstein, Eur. J. Immunol. 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (i.e., reactivity with the **complex** and/or component of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A

preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Detailed Description Text - DETX (48):

This example illustrates the recruitment of NF.kappa.B into a protein **complex (the IKK** signalsome) containing I.kappa.B kinase and other signaling proteins.

Detailed Description Text - DETX (50):

As shown in FIG. 1A, I.kappa.B.alpha. in cell extracts from unstimulated cells eluted with an apparent molecular weight of .about.300 kDa (lanes 5-7), consistent with the chromatographic properties of the inactive NF.kappa.B-I.kappa.B **complex** (Baeuerle and Baltimore, Genes Dev. 3:1689-98, 1989). In contrast, phosphorylated I.kappa.B.alpha. (from cells stimulated for periods too short to permit complete degradation of the protein) migrated at .about.600 kDa on the same chromatography columns (lanes 2, 3). This difference in migration was specific for I.kappa.B, since analysis of the same fractions indicated that the Jun N-terminal kinases JNK1 and JNK2 migrated with low apparent molecular weight and showed no difference in chromatographic behavior between stimulated and unstimulated cells. Stimulation-dependent recruitment of I.kappa.B into this larger protein **complex** corresponded with the appearance of phosphorylated I.kappa.B, suggesting that the **complex** contained the specific I.kappa.B kinases that mediate I.kappa.B phosphorylation. These results demonstrate that that NF.kappa.B activation involves recruitment into a protein **complex (the IKK** signalsome) containing I.kappa.B kinase and other signaling proteins.

Detailed Description Text - DETX (58):

This Example illustrates an alternate preparation of an **IKK** signalsome, and the characterization of the **complex**.

Detailed Description Text - DETX (84):

Of a large panel of antibodies tested, one of three anti-MKP-1 antibodies efficiently co-immunoprecipitated an inducible I.kappa.B kinase activity from HeLa cells as well as primary human umbilical vein endothelial cells (HUVEC). The co-immunoprecipitated kinase (**IKK** signalsome kinase) was inactive in unstimulated HeLa cells, but was rapidly activated within minutes of TNF.alpha. stimulation (FIG. 8A, top panel). The **IKK** signalsome kinase did not phosphorylate a mutant GST-I.kappa.B.alpha. protein in which serine residues 32 and 36 had been mutated to threonine (FIG. 8A top panel, even-numbered lanes). Activation of the signalsome kinase was maximal at 5 minutes and

declined thereafter, a time course consistent with the time course of I.kappa.B.alpha. phosphorylation and degradation under the same conditions (FIG. 8A, bottom panel). As expected, the signalsome I.kappa.B kinase was also activated by stimulation of cells with IL-1 or PMA (FIG. 8B, lanes 1-4); moreover, its activity was inhibited in cells treated with TPCK, a known inhibitor of NF.kappa.B activation (FIG. 8B, lane 7). Additionally, the **IKK** signalsome kinase specifically phosphorylated full-length wild-type I.kappa.B.alpha., but not a mutant I.kappa.B.alpha. bearing the serine 32, 36 to alanine mutations, in the context of a physiological RelA-I.kappa.B.alpha. **complex** (FIG. 8C, lanes 3, 4). Together these results indicate that the anti-MKP-1 antibody co-immunoprecipitated the **IKK** signalsome. The signalsome-associated I.kappa.B kinase met all the criteria expected of the authentic I.kappa.B kinase and had no detectable I.kappa.B.alpha. C-terminal kinase activity.

Detailed Description Text - DETX (99):

This example illustrates the absence of detectable free ubiquitin with a **IKK** signalsome prepared as in Example 3. Standard western blot procedures were performed (Amersham Life Science protocol, Arlington Heights, Ill.). 100 ng ubiquitin, 10 ng ubiquitin and 20 ul purified IkB.alpha. kinase **complex** was subjected to 16% Tricine SDS-PAGE (Novex, San Diego, Calif.), transferred to Hybond ECL Nitrocellulose membrane (Amersham Life Science, Arlington Heights, Ill.), and probed with antibodies directed against ubiquitin (MAB 1510; Chemicon, Temecula, Calif.). The results are shown in FIG. 10. Free ubiquitin could not be detected in the purified IkB.alpha. kinase preparation (even at very long exposures). The complexes described herein do not require addition of endogenous ubiquitin to detect IkB.alpha. kinase activity, nor is free ubiquitin a component in the purified IkB.alpha. kinase preparations of the present invention.

Detailed Description Text - DETX (113):

Both **IKK-1 and IKK-2** kinases were active when expressed in wheat germ extracts, since they were capable of autophosphorylation, but they were inactive with respect to phosphorylation of I.kappa.B substrates. Since both autophosphorylation and substrate phosphorylation were intact in rabbit reticulocyte lysates, there appeared to be a direct correlation between the association of **IKK-1 and IKK-2** into a higher order protein **complex** and the presence of specific I.kappa.B kinase activity in **IKK-1 and IKK-2** immunoprecipitates. This higher order **complex** is most likely the **IKK** signalsome itself. Indeed, immunoprecipitation of rabbit reticulocyte lysates with anti-MKP-1 antibody pulls down a low level of active I.kappa.B kinase activity characteristic of the **IKK** signalsome.

Detailed Description Text - DETX (114):

It is clear that the **IKK** signalsome contains multiple protein components in addition to **IKK-1 and IKK-2** (FIG. 11B). Some of these may be upstream kinases

such as MEKK-1 (Chen et al., Cell 84:853-62, 1996) or NIK (Malinin, et al., Nature 385:540-44, 1997); others may be adapter proteins that mediate the **IKK**-1:**IKK**-2 interaction. Indeed MEKK-1 copurifies with **IKK** signalsome activity (FIG. 1C), and two other signalsome proteins have been functionally identified. The protein crossreactive with anti-MKP-1 is an intrinsic component of the **IKK** signalsome kinases, since the I.kappa.B kinase activity coprecipitated with this antibody is stable to washes with 2-4 M urea. Moreover, both **IKK-1** immunoprecipitates and MKP-1 immunoprecipitates containing the **IKK** signalsome (FIG. 8C) contain an inducible RelA kinase whose kinetics of activation parallel those of the I.kappa.B kinase in the same immunoprecipitates. Another strong candidate for a protein in the signalsome **complex** is the E3 ubiquitin ligase that transfers multiubiquitin chains to phosphorylated I.kappa.B (Hershko et al., Annu. Rev. Biochem. 61:761-807, 1992).

Detailed Description Text - DETX (115):

These results indicate that **IKK-1 and IKK-2** are functional kinases within the **IKK** signalsome, which mediate I.kappa.B phosphorylation and NF.kappa.B activation. Appropriate regulation of **IKK-1 and IKK-2** may require their assembly into a higher order protein **complex,** which may be a heterodimer facilitated by adapter proteins, the complete **IKK** signalsome, or some intermediate subcomplex that contains both **IKK-1 and IKK-2**.

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PARENT-CASE:

This application is based on, and claims the benefit of, U.S. Provisional application Ser. No. 60/061,470, filed Oct. 9, 1997, the entire contents of which is herein incorporated by reference.

US-CL-CURRENT: 435/325, 435/194 , 435/252.3 , 435/320.1 , 536/23.2

ABSTRACT:

The present invention provides an isolated nucleic acid molecules encoding IKB kinase (**IKK**) catalytic subunit polypeptides, which are associated with an **IKK** serine protein kinase that phosphorylates a protein (IKB) that inhibits the activity of the NF-KB transcription factor, vectors comprising such nucleic acid molecules and host cells containing such vectors. In addition, the invention provides nucleotide sequences that can bind to a nucleic acid molecule of the invention, such nucleotide sequences being useful as probes or as antisense molecules. The invention also provides isolated **IKK** catalytic subunits, which can phosphorylate an IKB protein, and peptide portions of such **IKK** subunit. In addition, the invention provides anti-**IKK** antibodies, which specifically bind to an **IKK complex or an IKK** catalytic subunit, and **IKK**-binding fragments of such antibodies. The invention further provides methods of substantially purifying an **IKK complex,** methods of identifying an agent that can alter the association of an **IKK complex or an IKK** catalytic subunit with a second protein, and methods of identifying proteins that can interact with an **IKK complex or an IKK** catalytic subunit.

11 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC ---------

Abstract Text - ABTX (1):

The present invention provides an isolated nucleic acid molecules encoding IKB kinase (**IKK**) catalytic subunit polypeptides, which are associated with an **IKK** serine protein kinase that phosphorylates a protein (IKB) that inhibits the activity of the NF-KB transcription factor, vectors comprising such nucleic acid molecules and host cells containing such vectors. In addition, the invention provides nucleotide sequences that can bind to a nucleic acid molecule of the invention, such nucleotide sequences being useful as probes or as antisense molecules. The invention also provides isolated **IKK** catalytic subunits, which can phosphorylate an IKB protein, and peptide portions of such **IKK** subunit. In addition, the invention provides anti-**IKK** antibodies, which specifically bind to an **IKK complex or an IKK** catalytic subunit, and **IKK**-binding fragments of such antibodies. The invention further provides methods of substantially purifying an **IKK complex,** methods of identifying an agent that can alter the association of an **IKK complex or an IKK** catalytic subunit with a second protein, and methods of identifying proteins that can interact with an **IKK complex or an IKK** catalytic subunit.

Brief Summary Text - BSTX (15):

The present invention also provides isolated full length human **IKK** subunits, which can phosphorylate an IKB protein. For example, the invention provides an IKK.alpha. polypeptide having the amino acid sequence shown as SEQ ID NO: 2, particularly the amino acid sequence comprising amino acids 1 to 31 at the N-terminus of the polypeptide of SEQ ID NO: 2. In addition, the invention provides an IKK.beta. polypeptide having the amino acid sequence shown as SEQ ID NO: 15. The invention also provides peptide portions of an **IKK** subunit, including, for example, peptide portions comprising one or more contiguous amino acids of the N-terminal amino acids shown as residues 1 to 31 in SEQ ID NO: 2. A peptide portion of an **IKK** subunit can comprise the kinase domain of the **IKK** subunit or can comprise a peptide useful for eliciting production of an antibody that specifically binds to an IKB kinase or to the **IKK** subunit. Accordingly, the invention also provides anti-**IKK** antibodies that specifically bind to an **IKK complex** comprising an **IKK** subunit, particularly to the **IKK** subunit, for example, to an epitope comprising at least one of the amino acids shown as residues 1 to 31 of SEQ ID NO: 2, and also provides **IKK** subunit-binding fragments of such antibodies. In addition, the invention provides cell lines producing anti-**IKK** antibodies or **IKK**-binding fragments thereof.

Brief Summary Text - BSTX (16):

The invention also provides isolated IKB kinase complexes. As disclosed herein, an **IKK complex** can have an apparent molecular mass of about 900 kDa or about 300 kDa. An **IKK complex** is characterized, in part, in that it comprises an IKK.alpha. subunit, an IKK.beta. subunit, or both and can phosphorylate an IKB protein.

Brief Summary Text - BSTX (17):

The present invention further provides methods for isolating an **IKK complex or an IKK** subunit, as well as methods of identifying an agent that can alter the association of an **IKK complex or an IKK** subunit with a second protein that associates with the **IKK** in vitro or in vivo. Such a second protein can be, for example, another **IKK** subunit; an IKB protein, which is a substrate for **IKK** activity and is involved in a signal transduction pathway that results in the regulated expression of a gene; a protein that is upstream of the IKB kinase in a signal transduction pathway and regulates **IKK** activity; or a protein that acts as a regulatory subunit of the IKB kinase or of an **IKK** subunit and is necessary for full activation of the **IKK complex**. An agent that alters the association of an **IKK** subunit with a second protein can be, for example, a peptide, a polypeptide, a peptidomimetic or a small organic molecule. Such agents can be useful for modulating the level of phosphorylation of IKB in a cell, thereby modulating the activity of NF-KB in the cell and the expression of a gene regulated by NF-KB.

Brief Summary Text - BSTX (18):

The invention also provides methods of identifying proteins that can interact with an IKB kinase, including with an **IKK** subunit, such proteins which can be a downstream effector of the **IKK** such as a member of the IKB family of proteins or an upstream activator or a regulatory subunit of an **IKK**. Such proteins that interact with an **IKK complex or the IKK** subunit can be isolated, for example, by coprecipitation. with the **IKK** or by using the **IKK** subunit as a ligand, and can be involved, for example, in tissue specific regulation of NF-KB activation and consequent tissue specific gene expression.

Drawing Description Text - DRTX (2):

FIG. 1 shows a nucleotide sequence (SEQ ID NO: 1; lower case letter) and deduced amino acid sequence (SEQ ID NO: 2; upper case letters) of full length human IKK.alpha. subunit of an **IKK complex**. Nucleotide positions are indicated to the right and left of the sequence; the "A" of the ATG encoding the initiator methionine is shown as position 1. Underlined amino acid residues indicate the peptide portions of the protein ("peptide 1" and "peptide 2") that were sequenced and used to design oligonucleotide probes. The asterisk indicates the sequence encoding the STOP codon.

Detailed Description Text - DETX (2):

The present invention provides isolated nucleic acid molecules encoding polypeptide subunits of human serine protein kinase **complex,** the IKB kinase (**IKK**), which is activated in response to proinflammatory signals and phosphorylates proteins (IKB's) that bind to and inhibit the activity of NF-KB transcription factors. For example, the invention provides an isolated nucleic acid molecule (SEQ ID NO: 1) encoding a full length human IKK.alpha. subunit having the amino acid sequence shown as SEQ ID NO: 2 (FIG. 1). In addition, the invention provides an isolated nucleic acid molecule (SEQ ID NO: 14; FIG. 2) encoding a full length human IKK.beta. subunit having the amino acid sequence shown as SEQ ID NO: 15 (FIG. 3).

Detailed Description Text - DETX (4):

IKK.alpha. and IKK.beta. have been designated **IKK** subunits because they are components of an approximately 900 kDa **complex** having IKB kinase (**IKK**) activity and because they share substantial nucleotide and amino acid sequence homology. As disclosed herein, IKK.alpha. and IKK.beta. are related members of a family of **IKK** catalytic subunits (see FIG. 3). The 900 kDa IKB kinase **complex** can be isolated in a single step, for example, by immunoprecipitation using an antibody specific for an **IKK** subunit or by using metal ion chelation chromatography methods (see Example IV). A 300 kDa **IKK complex** also can be isolated as disclosed herein and has kinase activity for an IKB substrate (see Example III).

Detailed Description Text - DETX (43):

The present invention provides an isolated IKB kinase (**IKK**), including isolated full length **IKK** catalytic subunits. For example, the invention provides an isolated 300 kDa or 900 kDa **complex,** which comprises an IKK.alpha. or an IKK.beta. subunit and has IKB kinase activity (see Examples I, III and IV). In addition, the invention provides is an isolated human IKK.alpha. catalytic subunit (SEQ ID NO: 2; Example II), which contains a previously undescribed N-terminal amino acid sequence and essentially the C-terminal region of human CHUK (Connelly and Marcu, supra, 1995) and phosphorylates IKB.alpha. on Ser-32 and Ser-36 and IKB.beta. on Ser-19 and Ser-23 (DiDonato et al., supra, 1996; see, also, Regnier et al., supra, 1997). The invention also provides an isolated IKK.beta. catalytic subunit (SEQ ID NO: 15; Example III), which shares greater than 50% amino acid sequence identity with IKK.alpha., including conserved homology in the kinase domain, helix-loop-helix domain and leucine zipper domain.

Detailed Description Text - DETX (44):

As used herein, the term "isolated," when used in reference to an IKB kinase **complex or to an IKK** catalytic subunit of the invention, means that the **complex** or the subunit is relatively free from contaminating lipids, proteins, nucleic acids or other cellular material normally associated with an **IKK** in a cell. An isolated 900 kDa IKB kinase **complex** or 300 kDa **complex** can be isolated, for

example, by immunoprecipitation using an antibody that binds to an **IKK** catalytic subunit (see Examples III and IV). In addition, an isolated **IKK** subunit can be obtained, for example, by expression of a recombinant nucleic acid molecule such as SEQ ID NO: 1 or SEQ ID NO: 14, or can be isolated from a cell by a method comprising affinity chromatography using ATP or IKB as ligands (Example I) or using an anti-**IKK** subunit antibody. An isolated **IKK complex or IKK** subunit comprises at least 30% of the material in a sample, generally about 50% or 70% or 90% of a sample, and preferably about 95% or 98% of a sample, as described above with respect to nucleic acids.

Detailed Description Text - DETX (52):

A peptide portion of an **IKK** subunit can comprise the kinase domain of the **IKK** subunit and, therefore, can have the ability to phosphorylate an IKB protein. For example, a peptide portion of SEQ ID NO: 2 comprising amino acids 15 to 301 has the characteristics of a serine-threonine protein kinase domain (Hanks and Quinn, Meth. Enzymol. 200:38-62 (1991), which is incorporated herein by reference). Such a peptide portion of an **IKK** subunit can be examined for kinase activity by determining that it can phosphorylate IKB.alpha. at Ser-32 and Ser-36 or IKB.beta. at Ser-19 and Ser-23, using methods as disclosed herein. In addition, a peptide portion of an **IKK** subunit can comprise an immunogenic amino acid sequence of the polypeptide and, therefore, can be useful for eliciting production of an antibody that can specifically bind the **IKK** subunit or to an **IKK complex** comprising the subunit, particularly to an epitope comprising amino acid residue 30 as shown in SEQ ID NO: 2 or to an epitope of SEQ ID NO: 15, provided said epitope is not present in a CHUK protein. Accordingly, the invention also provides anti-**IKK** antibodies, which specifically bind to an epitope of an **IKK complex, particularly an IKK** catalytic subunit, and to **IKK** subunit binding fragments of such antibodies. In addition, the invention provides cell lines producing anti-**IKK** antibodies or **IKK**-binding fragments of such antibodies.

Detailed Description Text - DETX (53):

As used herein, the term "antibody" is used in its broadest sense to include polyclonal and monoclonal antibodies, as well as antigen binding fragments of such antibodies. With regard to an anti-**IKK** antibody of the invention, the term "antigen" means an **IKK** catalytic subunit protein, polypeptide or peptide portion thereof, or an **IKK complex** comprising an **IKK** catalytic subunit protein, polypeptide or peptide portion thereof. Thus, it should be recognized that, while an anti-**IKK** antibody can bind to and, for example, immunoprecipitate an **IKK complex,** the antibody specifically binds an epitope comprising at least a portion of an **IKK** catalytic subunit. An antibody of the invention also can be used to immunoprecipitate an **IKK** subunit, free of the **IKK complex**.

Detailed Description Text - DETX (56):

An anti-**IKK** antibody of the invention can be raised using an isolated **IKK** subunit or a peptide portion thereof and can bind to a free, uncomplexed form

of **IKK** subunit or can bind to **IKK** subunit when it is associated with a 300 kDa or 900 kDa **IKK complex**. In addition, an anti-**IKK** antibody of the invention can be raised against an isolated 300 kDa or 900 kDa IKB kinase **complex,** which can be obtained as disclosed herein. For convenience, an antibody of the invention is referred to generally herein as an "anti-IKB kinase antibody" or an "anti-**IKK** antibody." However, the skilled recognize that the various antibodies of the invention will have unique antigenic specificities, for example, for a free or complexed **IKK** subunit, or both, or for a 300 kDa or 900 kDa IKB kinase **complex,** or both.

Detailed Description Text - DETX (58):

Particularly useful antibodies of the invention include antibodies that bind with the free, but not the complexed, form of an **IKK** subunit or, alternatively, with the complexed, but not free, form of an **IKK** subunit. Antibodies of the invention also include antibodies that bind with the 300 kDa IKB kinase **complex** or the 900 kDa IKB kinase **complex** or both. It should be recognized, however, that an antibody specific for the 300 kDa or 900 kDa IKB kinase **complex** need not recognize an **IKK** subunit epitope in order to be encompassed within the claimed invention, since, prior to the present disclosure, the 300 kDa and 900 kDa **IKK** complexes were not known (see DiDonato et al., Nature 388:548-554 (1997)).

Detailed Description Text - DETX (59):

Antibodies of the invention that bind to an activated **IKK** but not to an inactive **IKK,** and, conversely, those that bind to an inactive form of the kinase but not to the activated form also are particularly useful. For example, an **IKK** can be activated by phosphorylation of an **IKK** subunit and, therefore, an antibody that recognizes the phosphorylated form of the **IKK,** but that does not bind to the unphosphorylated form can be obtained. In addition, **IKK** can be activated by release of a regulatory subunit and, therefore, an antibody that recognizes a form of the **IKK complex** that is not bound to the regulatory subunit can be obtained. Such antibodies are useful for identifying the presence of active **IKK** in a cell.

Detailed Description Text - DETX (61):

A kit incorporating an anti-**IKK** antibody, which can be specific for the active or inactive form of IKB kinase or can bind to an **IKK complex or to an IKK** subunit, regardless of the activity state, can be particularly useful. Such a kit can contain, in addition to an anti-**IKK** antibody, a reaction cocktail that provides the proper conditions for performing the assay, control samples that contain known amounts of an **IKK or IKK** subunit and, if desired, a second antibody specific for the anti-**IKK** antibody. Such an assay also should include a simple method for detecting the presence or amount of an **IKK or an IKK** subunit in a sample that is bound to the anti-**IKK** antibody.

Detailed Description Text - DETX (64):

Methods for raising polyclonal antibodies, for example, in a rabbit, goat, mouse or other mammal, are well known in the art (see Example V). In addition, monoclonal antibodies can be obtained using methods that are well known and routine in the art (Harlow and Lane, supra, 1988). Essentially, spleen cells from a mouse immunized with an **IKK complex or an IKK** subunit or peptide portion thereof can be fused to an appropriate myeloma cell line such as SP/02 myeloma cells to produce hybridoma cells. Cloned hybridoma cell lines can be screened using a labeled **IKK** subunit to identify clones that secrete anti-**IKK** monoclonal antibodies. Hybridomas expressing anti-**IKK** monoclonal antibodies having a desirable specificity and affinity can be isolated and utilized as a continuous source of the antibodies, which are useful, for example, for preparing standardized kits as described above. Similarly, a recombinant phage that expresses, for example, a single chain anti-**IKK** also provides a monoclonal antibody that can used for preparing standardized kits.

Detailed Description Text - DETX (66):

The present invention further provides methods of identifying an agent that can alter the association of an **IKK** catalytic subunit with a second protein, which can be an upstream activator, a downstream effector such as IKB, an interacting regulatory protein of the **IKK** subunit, or an interacting subunit associated with the 300 kDa or 900 kDa IKB kinase **complex**. As used herein, the term "associate" or "association," when used in reference to an **IKK** subunit and a second protein means that the **IKK** subunit and the second protein have a binding affinity for each other such that they form a bound **complex** in vivo or in vitro, including in a cell in culture or in a reaction comprising substantially purified reagents. For convenience, the term "bind" or "interact" is used interchangeably with the term "associate."

Detailed Description Text - DETX (67):

The affinity of binding of an **IKK** subunit and a second protein such as an IKB or another **IKK** subunit or other subunit present in an **IKK complex** is characterized in that it is sufficiently specific such that a bound **complex** can form in vivo in a cell or can form in vitro under appropriate conditions as disclosed herein. The formation or dissociation of a bound **complex** can be identified, for example, using the two hybrid assay or demonstrating communoprecipitation of the second protein with the **IKK** subunit, as disclosed herein, or using other well known methods such as equilibrium dialysis. Methods for distinguishing the specific association of an **IKK** subunit and a second protein from nonspecific binding to the **IKK** subunit are known in the art and, generally, include performing the appropriate control experiments to demonstrate the absence of nonspecific protein binding.

Detailed Description Text - DETX (68):

As used herein, the term "second protein" refers to a protein that

specifically associates with an **IKK** subunit ("first protein"). Such a second protein is exemplified herein by IKB proteins, including IKB.alpha. and IKB.beta., which are substrates for IKB kinase activity and are downstream of the IKB kinase in a signal transduction pathway that results in the regulated expression of a gene. In addition, such second proteins are exemplified by the proteins that, together with the **IKK** subunits, form a 300 kDa or 900 kDa IKB kinase **complex,** which coimmunoprecipitates using an anti-**IKK** antibody (see Example IV). Furthermore, since **IKK** subunits such as IKK.alpha. and IKK.beta. interact with each other to form homodimers or heterodimers, a second protein also can be a second **IKK** subunit, which can be the same as or different from the "first" protein.

Detailed Description Text - DETX (70):

In addition, a second protein can be a protein that is upstream of IKB kinase in a signal transduction pathway and associates with the **IKK complex, particularly with an IKK** catalytic subunit of the **IKK complex**. Such a second protein, which can be an upstream activator of the IKB kinase, can be identified using routine methods for identifying protein-protein interactions as disclosed herein. Such second proteins can be, for example, MEKK1 or PKR or CKII, each of which has been reported to be involved in a pathway leading to phosphorylation of IKB and activation of NF-KB, but neither of which has the characteristics expected of the common IKB kinase present at the point where the various NF-KB activation pathways converge (see, for example, Lee et al., supra, 1997), or can be the NF-KB-inducing kinase (NIK), which reportedly is upstream from **IKK** in an NF-KB activation pathway (Regnier et al., supra, 1997; Malinin et al., Nature 385:540-544 (1997)).

Detailed Description Text - DETX (71):

A second protein also can be a regulatory protein, which associates with an **IKK** catalytic subunit in an **IKK complex,** either constitutively as part of a 300 kDa or 900 kDa **complex** or in response to activation of a pathway leading to **IKK** activation. Such a regulatory protein can inhibit or activate **IKK** activity depending, for example, on whether the regulatory protein is associated with **IKK** and whether the regulatory protein associates with an **IKK** catalytic subunit in a free form or as part of an **IKK complex**. The regulatory protein also can be important for "docking" a catalytic **IKK** subunit to its substrate. The ability of a regulatory protein to associate with or dissociate from an **IKK** subunit or **IKK complex** can depend, for example, on the relative phosphorylation state of the regulatory protein. It is recognized that an upstream activator of **IKK** also can interact with such a regulatory protein, thereby indirectly inhibiting or activating the **IKK**.

Detailed Description Text - DETX (72):

As disclosed herein, two copurifying proteins were isolated by ATP and IKB affinity chromatography and identified by SDS-PAGE (Example I). Partial amino acid sequences were determined and cDNA molecules encoding the proteins were

obtained (see Examples I, II and III). One of the proteins has an apparent molecular mass of 85 kDa. Expression in a cell of a cDNA molecule encoding the 85 kDa protein resulted in increased NF-KB activity following cytokine induction as compared to control cells, whereas expression of the antisense of this cDNA decreased the basal NF-KB activity in the cells and prevented cytokine induction of NF-KB activity. Immunoprecipitation of the 85 kDa protein resulted in isolation of the **IKK complex,** the kinase activity of which was stimulated rapidly in response to TNF or to IL-1. Based on these functional analyses, the 85 kDa protein was determined to be a component of the 900 kDa IKB kinase **complex** and has been designated IKK.alpha. (SEQ ID NO: 2). The second protein, which copurified with the 85 kDa IKB kinase, has an apparent molecular mass of 87 kDa and shares greater than 50% amino acid sequence identity with IKK.alpha. and has been designated IKK.beta. (SEQ ID NO: 15).

Detailed Description Text - DETX (74):

A screening assay of the invention provides a means to identify an agent that alters the association of an **IKK complex or an IKK** catalytic subunit with a second protein such as the regulatory subunits discussed above. As used herein, the term "modulate" or "alter" when used in reference to the association of an **IKK** and a second protein, means that the affinity of the association is increased or decreased with respect to a steady state, control level of association, i.e., in the absence of an agent. Agents that can alter the association of an **IKK** with a second protein can be useful for modulating the level of phosphorylation of IKB in a cell, which, in turn, modulates the activity of NF-KB in the cell and the expression of a gene regulated by NF-KB. Such an agent can be, for example, an anti-idiotypic antibody as described above, which can inhibit the association of an **IKK** and IKB. A peptide portion of IKB.alpha. comprising amino acids 32 to 36, but containing substitutions for Ser-32 and Ser-36, is another example of such an agent, since the peptide can compete with IKB.alpha. binding to **IKK,** as is the corresponding peptide of IKB.beta..

Detailed Description Text - DETX (75):

A screening assay of the invention also is useful for identifying agents that directly alter the activity of an **IKK**. While such an agent can act, for example, by altering the association of an **IKK complex or IKK** catalytic subunit with a second protein, the agent also can act directly as a specific activator or inhibitor of **IKK** activity. Specific protein kinase inhibitors include, for example, staurosporin, the heat stable inhibitor of cAMP-dependent protein kinase, and the MLCK inhibitor, which are known in the art and commercially available. A library of molecules based, generally, on such inhibitors or on ATP or adenosine can be screened using an assay of the invention to obtain agents that desirably modulate the activity of an **IKK complex or an IKK** subunit.

Detailed Description Text - DETX (77):

A screening assay of the invention is particularly useful to identify, from among a diverse population of molecules, those agents that modulate the association of an **IKK complex or an IKK** catalytic subunit and another protein (referred to herein as a "second protein") or that directly alter the activity of **IKK**. Methods for producing libraries containing diverse populations of molecules, including chemical or biological molecules such as simple or **complex** organic molecules, peptides, proteins, peptidomimetics, glycoproteins, lipoproteins, polynucleotides, and the like, are well known in the art (Huse, U.S. Pat. No. 5,264,563, issued Nov. 23, 1993; Blondelle et al., Trends Anal. Chem. 14:83-92 (1995); York et al., Science 274:1520-1522 (1996); Gold et al., Proc. Natl. Acad. Sci. USA 94:59-64 (1997); Gold, U.S. Pat. No. 5,270,163, issued Dec. 14, 1993). Such libraries also can be obtained from commercial sources.

Detailed Description Text - DETX (79):

A drug screening assay of the invention utilizes an **IKK complex,** which can be isolated as disclosed herein; or an **IKK** subunit, which can be expressed, for example, from a nucleic acid molecule encoding the amino acid sequence shown in SEQ ID NO: 2 or in SEQ ID NO: 15; or can be purified as disclosed herein; or can utilize an **IKK** subunit fusion protein such as an IKK.alpha.-glutathione-S-transferase (GST) or IKK.beta.-histidine.sub.6 (HIS6) fusion protein, wherein the GST or HIS6 is linked to the **IKK** subunit and comprises a tag (see Example VI). The **IKK or IKK** subunit fusion protein is characterized, in part, by having an affinity for a solid substrate as well as having the ability to specifically associate with an appropriate second protein such as an IKB protein. For example, when an **IKK** catalytic subunit is used in a screening assay, the solid substrate can contain a covalently attached anti-**IKK** antibody, provided that the antibody binds the **IKK** subunit without interfering with the ability of the **IKK** subunit to associate with the second protein. Where an IKK.alpha.-GST fusion protein, for example, is used in such a screening assay, the solid substrate can contain covalently attached glutathione, which is bound by the GST tag component of the fusion protein. If desired, the **IKK** subunit or **IKK** subunit fusion protein can be part of an **IKK complex** in a drug screening assay of the invention.

Detailed Description Text - DETX (80):

A drug screening assay to identify an agent that alters the association of an **IKK complex or an IKK** subunit and a second protein can be performed by allowing, for example, the **IKK complex or IKK** subunit, which can be a fusion protein, to bind to the solid support, then adding the second protein, which can be an IKB such as IKB.alpha., and an agent to be tested, under conditions suitable for the association of the **IKK** and IKB.alpha. in the absence of a drug (see Example VI). As appropriate, the **IKK** can be activated or inactivated as disclosed herein and, typically, the **IKK** or the second protein is detectably labeled so as to facilitate identification of the association. Control reactions, which contain or lack either, the **IKK** component, or the IKB protein, or the agent, or which substitute the IKB protein with a second protein that is

known not to associate specifically with the **IKK,** also are performed. Following incubation of the reaction mixture, the amount of IKB.alpha. specifically bound to the **IKK** in the presence of an agent can be determined and compared to the amount of binding in the absence of the agent so that agents that modulate the association can be identified.

Detailed Description Text - DETX (81):

An **IKK** subunit such as IKK.alpha. or IKK.beta. used in a screening assay can be detectably labeled with a radionuclide, a fluorescent label, an enzyme, a peptide epitope or other such moiety, which facilitates a determination of the amount of association in a reaction. By comparing the amount of specific binding of an **IKK** subunit or an **IKK complex** and IKB in the presence of an agent as compared to the control level of binding, an agent that increases or decreases the binding of the **IKK** and the IKB can be identified. In comparison, where a drug screening assay is used to identify an agent that alters the activity of an **IKK,** the detectable label can be, for example, .gamma.-.sup.32 P-ATP, and the amount of .sup.32 P-IKB can be detected as a measure of **IKK** activity. Thus, the drug screening assay provides a rapid and simple method for selecting agents that desirably alter the association of an **IKK** and a second protein such as an IKB or for altering the activity of an **IKK**. Such agents can be useful, for example, for modulating the activity of NF-KB in a cell and, therefore, can be useful as medicaments for the treatment of a pathology due, at least in part, to aberrant NF-KB activity.

Detailed Description Text - DETX (84):

The invention also provides a method of obtaining an isolated **IKK complex or an IKK** catalytic subunit. For example, a 300 kDa or a 900 kDa **IKK complex,** comprising an IKK.alpha. subunit can be isolated from a sample by immunoprecipitation using an anti-IKK.alpha. antibody or by tagging the IKK.alpha. and using an antibody specific for the tag (see Examples III and IV). In addition, an **IKK** catalytic subunit can be isolated from a sample by 1) incubating the sample containing the **IKK** subunit with ATP, which is immobilized on a matrix, under conditions suitable for binding of the **IKK** subunit to the ATP; 2) obtaining from the immobilized ATP a fraction of the sample containing the **IKK** subunit; 3) incubating the fraction containing the **IKK** subunit with an IKB, which is immobilized on a matrix, under conditions suitable for binding of the **IKK** subunit to the IKB; and 4) obtaining from the immobilized IKB an isolated **IKK** catalytic subunit. Such a method of isolating an **IKK** subunit is exemplified herein by the use of ATP affinity chromatography and IKB.alpha. affinity chromatography to isolate IKK.alpha. or IKK.beta. from a sample of HeLa cells (see Example I).

Detailed Description Text - DETX (85):

The skilled artisan will recognize that a ligand such as ATP or an IKB or an anti-**IKK** antibody also can be immobilized on various other matrices, including, for example, on magnetic beads, which provide a rapid and simple method of

obtaining a fraction containing an ATP- or an IKB-bound **IKK complex or IKK** subunit or an anti-IKB kinase-bound **IKK** from the remainder of the sample. Methods for immobilizing a ligand such as ATP or an IKB or an antibody are well known in the art (Haystead et al., Eur. J. Biochem. 214:459-467 (1993), which is incorporated herein by reference; see, also, Hermanson, supra, 1996). Similarly, the artisan will recognize that a sample containing an **IKK complex or an IKK** subunit can be a cell, tissue or organ sample, which is obtained from an animal, including a mammal such as a human, and prepared as a lysate; or can be a bacterial, insect, yeast or mammalian cell lysate, in which an **IKK** catalytic subunit is expressed from a recombinant nucleic acid molecule. As disclosed herein, a recombinantly expressed IKK.alpha. or IKK.beta. such as a tagged IKK.alpha. or IKK.beta. associates into an active 300 kDa and 900 kDa **IKK complex** (see Examples III and IV).

Detailed Description Text - DETX (86):

The invention also provides a method of identifying a second protein that associates with an **IKK complex, particularly with an IKK** subunit. A transcription activation assay such as the yeast two hybrid system is particularly useful for the identification of protein-protein interactions (Fields and Song, Nature 340:245-246 (1989), which is incorporated herein by reference). In addition, the two hybrid assay is useful for the manipulation of protein--protein interaction and, therefore, also is useful in a screening assay to identify agents that modulate the specific interaction.

Detailed Description Text - DETX (89):

One adaptation of the transcription activation assay, the yeast two hybrid system, uses S. cerevisiae as a host cell for vectors that express the hybrid proteins. For example, a yeast host cell containing a reporter lacZ gene linked to a LexA operator sequence can be used to identify specific interactions between an **IKK** subunit and a second protein, where the DNA-binding domain is the LexA binding domain, which binds the LexA promoter, and the trans-activation domain is the B42 acidic region. When the LexA domain is bridged to the B42 transactivation domain through the interaction of the **IKK** subunit with a second protein, which can be expressed, for example, from a cDNA library, transcription of the reporter lacZ gene is activated. In this way, proteins that interact with the **IKK** subunit can be identified and their role in a signal transduction pathway mediated by the **IKK** can be elucidated. Such second proteins can include additional subunits comprising the 300 kDa or 900 kDa **IKK complex**.

Detailed Description Text - DETX (92):

An agent that alters the catalytic activity of an **IKK** or that alters the association of an **IKK** subunit or **IKK complex** and a second protein such as an IKB or an **IKK** regulatory subunit or an upstream activator of an **IKK** can be useful as a drug to reduce the severity of a pathology characterized by aberrant NF-KB activity. For example, a drug that increases the activity of an

IKK or that increases the affinity of an **IKK** catalytic subunit and IKB.alpha. can increase the amount of IKB.alpha. phosphorylated on Ser-32 or Ser-36 and, therefore, increase the amount of active NF-KB and the expression of a gene regulated by NF-KB, since the drug will increase the level of phosphorylated IKB.alpha. in the cell, thereby allowing NF-KB translocation to the nucleus. In contrast, a drug that decreases or inhibits the catalytic activity of an **IKK** or the association of an **IKK** catalytic subunit and IKB.alpha. can be useful where it is desirable to decrease the level of active NF-KB in a cell and the expression of a gene induced by activated NF-KB. It should be recognized that an antisense **IKK** subunit molecule of the invention also can be used to decrease **IKK** activity in a cell by reducing or inhibiting expression of the **IKK** subunit or by reducing or inhibiting its responsiveness to an inducing agent such as TNF.alpha., Il-1 or phorbol ester (see Example II). Accordingly, the invention also provides methods of treating an individual suffering from a pathology characterized by aberrant NF-KB activity by administering to the individual an agent that modulates the catalytic activity of an **IKK** or that alters the association of an **IKK** subunit and a second protein such as IKB or a subunit of a 300 kDa or 900 kDa **IKK complex** that interacts with the **IKK** subunit.

Detailed Description Text - DETX (94):

Glucocorticoids are potent anti-inflammatory and immunosuppressive agents that are used clinically to treat various pathologic conditions, including autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosis and asthma. Glucocorticoids suppress the immune and inflammatory responses, at least in part, by increasing the rate of IKB.alpha. synthesis, resulting in increased cellular levels of IKB.alpha., which bind to and inactivate NF-KB (Scheinman et al., Science 270:283-286 (1995); Auphan et al., Science 270:286-290 (1995)). Thus, glucocorticoids suppress NF-KB mediated expression of genes encoding, for example, cytokines, thereby suppressing the immune, inflammatory and acute phase responses. However, glucocorticoids and glucocorticoid-like steroids also are produced physiologically and are required for normal growth and development. Unfortunately, prolonged treatment of an individual with higher than physiological amounts of glucocorticoids produces clinically undesirable side effects. Thus, the use of an agent that alters the activity of an **IKK** or that alters the association of an **IKK complex or IKK** subunit and a second protein, as identified using a method of the invention, can provide a means for selectively altering NF-KB activity without producing some of the undesirable side effects associated with glucocorticoid treatment.

Detailed Description Text - DETX (97):

In addition, the decreased level of NF-KB activity may allow the virus-infected cell to undergo apoptosis, resulting in a decrease in the viral load in the individual. As such, it can be particularly useful to treat virus-infected cells ex vivo with an agent identified using a method of the invention. For example, peripheral blood mononuclear cells (PBMCs) can be collected from an HIV-1 infected individual and treated in culture with an agent that decreases the activity of an **IKK** or alters the association of an **IKK complex or an IKK** catalytic subunit with an IKB. Such a treatment can be

useful to purge the PBMCs of the virus-infected cells by allowing apoptosis to proceed. The purged population of PBMCs then can be expanded, if desired, and readministered to the individual.

Detailed Description Text - DETX (98):

Rel/NF-KB proteins also are involved in a number of different types of cancer. For example, the adhesion of cancer cells to endothelial cells is increased due to treatment of the cancer cells with IL-1, suggesting that NF-KB induced the expression of cell adhesion molecules, which mediated adherence of the tumor cells to the endothelial cells; agents such as aspirin, which decrease NF-KB activity, blocked the adhesion by inhibiting expression of the cell adhesion molecules (Tozawa et al., Cancer Res. 55:4162-4167 (1995)). These results indicate that an agent that decreases the activity of an **IKK** or that decrease the association of an **IKK** and IKB or of an **IKK** subunit and a second protein, for example, a second protein present in an **IKK complex,** can be useful for reducing the likelihood of metastasis of a tumor in an individual.

Detailed Description Text - DETX (102):

Identification and Characterization of a Human IKB Kinase **Complex and IKK** Subunits

Detailed Description Text - DETX (108):

B. Purification of **IKK Complex and IKK** Subunits

Detailed Description Text - DETX (122):

Since the 85 kDa IKK.alpha. band identified by the kinase assay following the above procedure contained only about 10% of the total purified protein, three additional criteria were used to confirm that the identified band was an intrinsic component of the **IKK complex**.

Detailed Description Text - DETX (161):

Similarly to the purified **IKK complex and the complex** associated with IKK.alpha., the IKK.beta. immune **complex** phosphorylated wt IKB.alpha. and IKB.beta., but not mutants in which the inducible phosphorylation sites (Ser-32 and Ser-36 for IKB.alpha. and Ser-19 and Ser-23 for IKB.alpha.) were replaced with either alanines or threonines. However, a low level of residual phosphorylation of full length IKB.alpha. (A32/A36) was observed due to phosphorylation of sites in the C-terminal portion of the protein (DiDonato et al., supra, 1997). Single substitution mutants, IKB.alpha. (A32) and IKB (A36), were phosphorylated almost as efficiently as wt IKB.alpha., indicating that IKK.beta.-associated **IKK** activity can phosphorylate IKB.alpha. at both Ser-32 and Ser-36.

Detailed Description Text - DETX (164):

As shown in Example I, IKK.alpha. and IKK.beta. copurified in about a 1:1 ratio through several chromatographic steps, suggesting that the two proteins interact with each other. The ability of the **IKK** subunits to interact in a functional **complex** and the effect of each subunit on the activity of the other subunit was examined using 293 cells transfected with expression vectors encoding Flag(M2)-IKK.alpha. or M2-IKK.alpha. and HA-IKK.beta., either alone or in combination (see Hopp et al., BioTechnology 6:1204-1210 (1988)). After 24 hr, samples of the cells were stimulated with TNF, lysates were prepared from stimulated and unstimulated cells, and one portion of the lysates was precipitated with anti-Flag antibodies (Eastman Kodak Co.; New Haven Conn.) and another portion was precipitated with anti-HA antibodies. The **IKK** activity associated with the different immune complexes and their content of IKK.alpha. and IKK.beta. were measured.

Detailed Description Text - DETX (167):

The HA-IKK.alpha.-associated **IKK** had a low level of basal specific activity, whereas expression of HA-IKK.beta. resulted in high basal specific activity that was increased when higher amounts of HA-IKK.beta. were expressed. However, the specific **IKK** activity associated with either IKK.alpha. or IKK.beta. isolated from TNF-stimulated cells was very similar and was not considerably affected by their expression level. These results indicate that titration of a negative regulator or formation of a constitutively active **IKK complex** can occur due to overexpression of IKK.beta..

Detailed Description Text - DETX (170):

Since the results described above revealed that HA-IKK.beta. associates with endogenous IKK.alpha. to generate a functional cytokine-regulated **IKK complex,** this association was examined further by transfecting HeLa cells with either empty expression vector or small amounts (1 .mu.g/60 mm plate) of either HA-IKK.alpha. or HA-IKK.beta. vectors. After 24 hr, samples of the transfected cell populations were stimulated with 20 ng/ml TNF for 5 min, then cell lysates were prepared and separated by gel filtration on a SUPEROSE 6 column. One portion of each column fraction was immunoprecipitated with a polyclonal antibody specific for IKK.alpha. and assayed for IKK.alpha.-associated **IKK** activity, while a second portion was precipitated with anti-HA antibody and examined for HA-IKK.beta.- or HA-IKK.alpha.-associated **IKK** activity. Relative specific activity was determined by immunoprecipitating the complexes, separating the proteins by SDS-PAGE, blotting the proteins onto IMOBILON membranes (Millipore; Bedford Mass.), immunoblotting with anti-HA antibody and quantitating the levels of IKB phosphorylation and HA-tagged proteins by phosphoimaging. The results demonstrated that endogenous IKK.alpha.-associated **IKK** activity exists as two complexes, a larger **complex** of approximately 900 kDa and a smaller one of approximately 300 kDa. Stimulation with TNF increased the **IKK** activity of both

complexes, although the extent of increase was considerably greater for the 900 kDa **complex**.

Detailed Description Text - DETX (171):

HA-IKK.beta.-associated **IKK** activity had exactly the same distribution as the IKK.alpha.-associated activity, eluting at 900 kDa and 300 kDa and, again, the extent of TNF responsiveness was considerably greater for the 900 kDa **complex**. Comparison to the IKK.alpha.-associated activity in cells transfected with the empty vector indicated that HA-IKK.beta. expression produced a modest, approximately 2-fold increase in the relative amount of **IKK** activity associated with the smaller 300 kDa **complex**. These results indicate that the 300 kDa **IKK complex,** like the 900 kDa **complex,** contains both IKK.alpha. and IKK.beta.. However, the 300 kDa lacks other subunits present in the 900 kDa **complex**. When IKK.beta. was overexpressed, the relative amount of the smaller **complex** increased, indicating that some of the subunits that are unique to the larger **complex** are present in a limited amount.

Detailed Description Text - DETX (202):

Alternatively, the labeled IKB or other appropriate second protein can be added to the immobilized **IKK** subunit and allowed to associate, then the agent can be added. Such a method allows the identification of agents that can induce the dissociation of a bound **complex** comprising the **IKK** subunit and IKB. Similarly, a screening assay of the invention can be performed using the 900 kDa **IKK complex,** comprising an **IKK** subunit.

US-PAT-NO: 6235513

DOCUMENT-IDENTIFIER: US 6235513 B1

TITLE: IKK-.alpha. proteins, nucleic acids and methods

DATE-ISSUED: May 22, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rothe; Mike	San Mateo	CA	N/A	N/A
Cao; Zhaodan	Pacifica	CA	N/A	N/A
Regnier; Catherine	South San Francisco	CA	N/A	N/A

APPL-NO: 09/ 023324

DATE FILED: February 13, 1998

PARENT-CASE:

This a divisional application of U.S. Ser. No. 08/890,854, filed Jul. 10, 1997, which is a continuing application under 35USC120 of U.S. Ser. No. 08/887,115 filed Jul. 1, 1997, abandoned.

US-CL-CURRENT: 435/194

ABSTRACT:

The invention provides methods and compositions relating to an I.kappa.B kinase, IKK-.alpha., and related nucleic acids. The polypeptides may be produced recombinantly from transformed host cells from the disclosed IKK-.alpha. encoding nucleic acids or purified from human cells. The invention provides isolated IKK-.alpha. hybridization probes and primers capable of specifically hybridizing with the disclosed IKK-.alpha. genes, IKK-.alpha.-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

36 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (7):

Here, we disclose a novel human kinase I.kappa.B Kinase, **IKK**-.alpha., as a NIK-interacting protein. **IKK**-.alpha. has sequence similarity to the

conceptual translate of a previously identified open reading frame (SEQ ID NO:5) postulated to encode a serine-threonine kinase of unknown function (`Conserved Helix-loop-helix Ubiquitous Kinase` or CHUK, Connelly and Marcu, 1995; Mock et al., 1995). Catalytically inactive mutants of **IKK**-.alpha. are shown to suppress NF-.kappa.B activation induced by TNF and IL-1 stimulation as well as by TRAF and NIK overexpression; transiently expressed **IKK**-.alpha. is shown to associate with the endogenous I.kappa.B.alpha. **complex; and** **IKK**-.alpha. is shown to phosphorylate I.kappa.B.alpha. on serines 32 and 36.

Brief Summary Text - BSTX (32):

The interaction of **IKK**-.alpha. with NIK was confirmed in mammalian cell coimmunoprecipitation assays. Human **IKK**-.alpha. containing an N-terminal Flag epitope tag was transiently coexpressed in 293 human embryonic kidney cells with Myc epitope-tagged NIK or HA epitope-tagged TRAF proteins. Cell lysates were immunoprecipitated using a monoclonal antibody against the Flag epitope, and coprecipitating NIK or TRAF proteins were detected by immunoblot analysis with an anti-Myc or anti-HA monoclonal antibodies. In this assay, **IKK**-.alpha. was able to coprecipitate NIK confirming the interaction between both proteins as detected for **IKK**-.alpha. by yeast two-hybrid analysis. Also, a deletion mutant **IKK**-.alpha. protein lacking most of the N-terminal kinase domain (**IKK**-.alpha..sub.(307-745)) was able to associate with NIK, indicating that the .alpha.-helical C-terminal half of **IKK**-.alpha. mediates the interaction with NIK. In contrast to NIK, **IKK**-.alpha. failed to associate with either TRAF2 or TRAF3. However, when NIK was coexpressed with **IKK**-.alpha. and TRAF2, strong coprecipitation of TRAF2 with **IKK**-.alpha. was detected, indicating the formation of a ternary **complex** between **IKK**-.alpha., NIK and TRAF2.

US-PAT-NO: 6235512

DOCUMENT-IDENTIFIER: US 6235512 B1

TITLE: IKK-.alpha. proteins, nucleic acids and methods

DATE-ISSUED: May 22, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rothe; Mike	San Mateo	CA	N/A	N/A
Cao; Zhaodan	Pacifica	CA	N/A	N/A
Regnier; Catherine	South San Francisco	CA	N/A	N/A

APPL-NO: 08/ 890854

DATE FILED: July 10, 1997

PARENT-CASE:

This a continuing application under 35USC120 of U.S. Ser. No. 08/887,115 filed Jul. 1, 1997, abandoned.

US-CL-CURRENT: 435/194, 435/325 , 536/23.2

ABSTRACT:

The invention provides methods and compositions relating to an I.kappa.B kinase, IKK-.alpha., and related nucleic acids. The polypeptides may be produced recombinantly from transformed host cells from the disclosed IKK-.alpha. encoding nucleic acids or purified from human cells. The invention provides isolated IKK-.alpha. hybridization probes and primers capable of specifically hybridizing with the disclosed IKK-.alpha. genes, IKK-.alpha.-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

17 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (7):

Here, we disclose a novel human kinase I.kappa.B Kinase, **IKK**-.alpha., as a NIK-interacting protein. **IKK**-.alpha. has sequence similarity to the conceptual translate of a previously identified open reading frame (SEQ ID

NO:5) postulated to encode a serine-threonine kinase of unknown function (`Conserved Helix-loop-helix Ubiquitous Kinase` or CHUK, Connelly and Marcu, 1995; Mock et al., 1995). Catalytically inactive mutants of **IKK**-.alpha. are shown to suppress NF-.kappa.B activation induced by TNF and IL-1 stimulation as well as by TRAF and NIK over expression; transiently expressed **IKK**-.alpha. is shown to associate with the endogenous I.kappa.B.alpha. **complex; and IKK**-.alpha. is shown to phosphorylate I.kappa.B.alpha. on serines 32 and 36.

Detailed Description Text - DETX (6):

The interaction of **IKK**-.alpha. with NIK was confirmed in mammalian cell coimmunoprecipitation assays. Human **IKK**-.alpha. containing an N-terminal Flag epitope tag was transiently coexpressed in 293 human embryonic kidney cells with Myc epitope-tagged NIK or HA epitope-tagged TRAF proteins. Cell lysates were immunoprecipitated using a monoclonal antibody against the Flag epitope, and coprecipitating NIK or TRAF proteins were detected by immunoblot analysis with an anti-Myc or anti-HA monoclonal antibodies. In this assay, **IKK**-.alpha. was able to coprecipitate NIK confirming the interaction between both proteins as detected for **IKK**-.alpha. by yeast two-hybrid analysis. Also, a deletion mutant **IKK**-.alpha. protein lacking most of the N-terminal kinase domain (**IKK**-.alpha..sub.(307-745)) was able to associate with NIK, indicating that the .alpha.-helical C-terminal half of **IKK**-.alpha. mediates the interaction with NIK. In contrast to NIK, **IKK**-.alpha. failed to associate with either TRAF2 or TRAF3. However, when NIK was coexpressed with **IKK**-.alpha. and TRAF2, strong coprecipitation of TRAF2 with **IKK**-.alpha. was detected, indicating the formation of a ternary **complex** between **IKK**-.alpha., NIK and TRAF2.

US-PAT-NO: 6235492

DOCUMENT-IDENTIFIER: US 6235492 B1

TITLE: IKK-.alpha. proteins, nucleic acids and methods

DATE-ISSUED: May 22, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rothe; Mike	San Mateo	CA	N/A	N/A
Cao; Zhaodan	Pacifica	CA	N/A	N/A
Regnier; Catherine	South San Francisco	CA	N/A	N/A

APPL-NO: 09/ 032476

DATE FILED: February 26, 1998

PARENT-CASE:

This is a divisional application of U.S. Ser. No. 08/890,854, filed Jul. 10, 1997, which is a continuing application under 35USC120 of U.S. Ser. No. 08/887,115 filed Jul. 1, 1997, abandoned.

US-CL-CURRENT: 435/15

ABSTRACT:

The invention provides methods and compositions relating to an I.kappa.B kinase, IKK-.alpha., and related nucleic acids. The polypeptides may be produced recombinantly from transformed host cells from the disclosed IKK-.alpha. encoding nucleic acids or purified from human cells. The invention provides isolated IKK-.alpha. hybridization probes and primers capable of specifically hybridizing with the disclosed IKK-.alpha. genes, IKK-.alpha.-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

49 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (8):

Here, we disclose a novel human kinase I.kappa.B Kinase, **IKK**-.alpha., as a NIK-interacting protein. **IKK**-.alpha. has sequence similarity to the

conceptual translate of a previously identified open reading frame (SEQ ID NO:5) postulated to encode a serine-threonine kinase of unknown function (`Conserved Helix-loop-helix Ubiquitous Kinase` or CHUK, Connelly and Marcu, 1995; Mock et al., 1995). Catalytically inactive mutants of **IKK**-.alpha. are shown to suppress NF-.kappa.B activation induced by TNF and IL-1 stimulation as well as by TRAF and NIK overexpression; transiently expressed **IKK**-.alpha. is shown to associate with the endogenous I.kappa.B.alpha. **complex; and** **IKK**-.alpha. is shown to phosphorylate I.kappa.B.alpha. on serines 32 and 36.

Brief Summary Text - BSTX (33):

The interaction of **IKK**-.alpha. with NIK was confirmed in mammalian cell coimmunoprecipitation assays. Human **IKK**-.alpha. containing an N-terminal Flag epitope tag was transiently coexpressed in 293 human embryonic kidney cells with Myc epitope-tagged NIK or HA epitope-tagged TRAF proteins. Cell lysates were immunoprecipitated using a monoclonal antibody against the Flag epitope, and coprecipitating NIK or TRAF proteins were detected by immunoblot analysis with an anti-Myc or anti-HA monoclonal antibodies. In this assay, **IKK**-.alpha. was able to coprecipitate NIK confirming the interaction between both proteins as detected for **IKK**-.alpha. by yeast two-hybrid analysis. Also, a deletion mutant **IKK**-.alpha. protein lacking most of the N-terminal kinase domain (**IKK**-.alpha..sub.(307-745)) was able to associate with NIK, indicating that the a-helical C-terminal half of **IKK**-.alpha. mediates the interaction with NIK. In contrast to NIK, **IKK**-.alpha. failed to associate with either TRAF2 or TRAF3. However, when NIK was coexpressed with **IKK**-.alpha. and TRAF2, strong coprecipitation of TRAF2 with **IKK**-.alpha. was detected, indicating the formation of a ternary **complex** between **IKK**-.alpha., NIK and TRAF2.

US-PAT-NO: 6232081

DOCUMENT-IDENTIFIER: US 6232081 B1

TITLE: Method for the detection of NF-.kappa.B regulatory factors

DATE-ISSUED: May 15, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Harper; Jeffrey Wade	Sugarland	TX	N/A	N/A
Elledge; Stephen J.	Houston	TX	N/A	N/A
Winston; Jeffrey T.	Sugar Land	TX	N/A	N/A

APPL-NO: 09/ 172841

DATE FILED: October 15, 1998

PARENT-CASE:

This application is a Continuation-in-Part Application of U.S. pat. appl. Ser. No. 08/951,621, filed Oct. 16, 1997, pending, which is hereby incorporated herein by reference in its entirety.

US-CL-CURRENT: 435/7.1, 435/7.2 , 436/501 , 436/516 , 436/536

ABSTRACT:

The present invention provides compositions and methods for gene identification, as well as drug discovery and assessment. In particular, the present invention provides components of an E3 complex involved in ubiquitination of cell cycle regulators and other proteins, as well as members of a class of proteins that directly function in recognition of ubiquitination targets. The present invention also provides sequences of multiple F-box proteins.

4 Claims, 33 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 15

---------- KWIC ---------

Detailed Description Text - DETX (188):

NF-.kappa.B activation involves a multi-step signal transduction pathway

(Baeuerle and Baltimore, supra) involving receptor activation, activation of kinases (IKK.alpha. and IKK.beta. that phosphorylate I.kappa.B (the endogenous inhibitor of NF-.kappa.B), ubiquitination of I.kappa.B, proteolysis of I.kappa.B, and translocation of NF-.kappa.B to the nucleus. Recent advances include identification of **IKKs** (DiDonato et al., Nature 388:548 [1997]; Mercurio et al., Science 278:860 [1997]; Regnier et al., Cell 90:373 [1997]; Woronicz et al., Science 278:866 [1997]; and Zandi et al., Nature 387:151 [1997]) and the components of the TNF receptor **complex** (reviewed by Tewari and Dixit, supra). In contrast, prior to the present invention, virtually nothing was known about the molecules that function in the ubiquitination step.

Detailed Description Text - DETX (203):

SEQ ID NO:60) will not inhibit I.kappa.B ubiquitination when added in vitro to a crude cell lysate which supports I.kappa.B ubiquitination in a manner that is dependent upon the phosphorylation of Ser-32 and Ser-36 in I.kappa.B. In contrast the same peptide that has been phosphorylated on Ser-32 and Ser-36 will block the ubiquitination of I.kappa.B. Similarly, phosphorylated I.kappa.B peptide will block nuclear translocation of NF-.kappa.B in intact cells in response to stimuli while the unphosphorylated peptide will not. It is known that I.kappa.B needs to be phosphorylated on these two serines by **IKK** for ubiquitination to occur and this phosphorylation serves as the signal. These phosphopeptides derived from I.kappa.B are thought to block I.kappa.B ubiquitination by competing with the full-length I.kappa.B substrate for the recognition factor of the ubiquitin ligase that is normally functioning in I.kappa.B ubiquitination. Thus, the finding of the present invention that this same phosphorylated I.kappa.B peptide, but not the unphosphorylated peptide, will specifically interact with the SCF slimb **complex** suggests that this slimb **complex** is the ubiquitin ligase for I.kappa.B. Thus, the present invention provides a novel E3 ubiquitin ligase **complex,** thereby providing means to identify therapeutic targets for regulating NF-.kappa.B activity, to identify the molecular determinants that confer the ability of this ligase to recognize phosphorylated I.kappa.B, and to identify molecules that can disrupt this interaction.

US-PAT-NO: 6214582

DOCUMENT-IDENTIFIER: US 6214582 B1

TITLE: Y2H35 a strong IKK binding protein

DATE-ISSUED: April 10, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Marcu; Kenneth B.	Stony Brook	NY	N/A	N/A

APPL-NO: 09/ 193266

DATE FILED: November 16, 1998

US-CL-CURRENT: 435/69.1, 435/252.3 , 435/320.1 , 435/325 , 435/6 , 536/23.1 , 536/23.5

ABSTRACT:

The present invention provides an isolated I.kappa.B kinase binding protein designated Y2H35 and functional equivalents therof. The amino acid sequence of Y2H35, the nucleotide sequence encoding Y2H35, and other related protein and nucleic acid molecules are also provided.

13 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Other Reference Publication - OREF (11):

Zandi et al., "The I.kappa.B Kinase **Complex (IKK**) Contains Two Kinase Subunits, IKK.alpha.and IKK.beta., Necessary for I.kappa.B Phosphorylation and NF-.kappa.B Activation", Cell 91: 243-252 (1997).

US-PAT-NO: 6172195

DOCUMENT-IDENTIFIER: US 6172195 B1

TITLE: IKAP proteins and methods

DATE-ISSUED: January 9, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cohen; Lucie	South San Francisco	CA	N/A	N/A
Baeuerle; Patrick	South San Francisco	CA	N/A	N/A

APPL-NO: 09/ 286891

DATE FILED: April 6, 1999

PARENT-CASE:

This is a divisional application of U.S. Ser. No. 08/971,244, filed Nov. 16, 1997, now U.S. Pat. No. 5,891,719, which is incorporated herein by reference.

US-CL-CURRENT: 530/350, 436/501 , 530/300 , 530/351

ABSTRACT:

The invention provides methods and compositions relating to IKAP proteins which regulate cellular signal transduction and transcriptional activation, and related nucleic acids. The polypeptides may be produced recombinantly from transformed host cells from the disclosed IKAP encoding nucleic acids or purified from human cells. The invention provides isolated IKAP hybridization probes and primers capable of specifically hybridizing with the disclosed IKAP genes, IKAP-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

26 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

---------- KWIC ---------

Brief Summary Text - BSTX (6):

The NF-.kappa.B-inducing kinase (NIK) is a member of the MAP kinase kinase

kinase (MAP3K) family that was identified as a TRAF2-interacting protein (Malinin et al., 1997). NIK activates NF-.kappa.B when overexpressed, and kinase-inactive mutants of NIK comprising its TRAF2-interacting C-terminal domain (NIK.sub.(624-947)) or lacking two crucial lysine residues in its kinase domain (NIK.sub.(KK429-430AA)) behave as dominant-negative inhibitors that suppress TNF-, IL-1-, and TRAF2-induced NF-.kappa.B activation (Malinin et al., 1997). Recently, NIK was found to associate with additional members of the TRAF family, including TRAF5 and TRAF6. Catalytically inactive mutants of NIK also inhibited TRAF5- and TRAF6-induced NF-.kappa.B activation, thus providing a unifying concept for NIK as a common mediator in the NF-.kappa.B signaling cascades triggered by TNF and IL-1 downstream of TRAFs. Recently two NIK-interacting protein designated characterized as novel human kinase I.kappa.B Kinases, **IKK**-.alpha. and IKK.beta. have been reported (Woronicz et al., 1997; Mercurio et al. 1997; Maniatis, 1997). Catalytically inactive mutants of **IKK** suppress NF-.kappa.B activation induced by TNF and IL-1 stimulation as well as by TRAF and NIK overexpression; transiently expressed **IKK** associates with endogenous I.kappa.B.alpha. **complex; and IKK** phosphorylates I.kappa.B.alpha. on serines 32 and 36.

US-PAT-NO: 6083732

DOCUMENT-IDENTIFIER: US 6083732 A

TITLE: Biologically active alternative form of the ikka.alpha. I.kappa.B kinase

DATE-ISSUED: July 4, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Marcu; Kenneth B.	Stony Brook	NY	N/A	N/A

APPL-NO: 09/ 160483

DATE FILED: September 25, 1998

US-CL-CURRENT: 435/194, 435/252.3 , 435/320.1 , 435/325 , 536/23.2

ABSTRACT:

The present invention provides an isolated I.kappa.B kinase designated IKK.alpha..DELTA.C, that regulates NF.kappa.B gene transcription. IKK.alpha..DELTA.C is an I.kappa.B protein kinase having a kinase domain, a leucine zipper like .alpha.-helix domain, and no helix-loop-helix domain. Also provided are the amino acid sequence of IKK.alpha..DELTA.C, the nucleotide sequence encoding IKK.alpha..DELTA.C, and other related protein and nucleic acid molecules. The invention provides antibodies specific for IKK.alpha..DELTA.C and methods of using the IKK.alpha..DELTA.C proteins of the invention in the development of antiinflammatory agents or immunosuppressants.

8 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------

Detailed Description Text - DETX (129):

In time course experiments shown in FIG. 3, the activation profiles of IKK.alpha. and IKK.alpha..DELTA.C in response to TNF.alpha. stimulation were found to be superimposable. 293 cells were transiently transfected with plasmid encoding HA-IKK.alpha. or HA-IKK.alpha..DELTA.C. Twenty fours hours after transfection, cells were stimulated or not for the times indicated with TNF.alpha. (10 ng/ml) prior to cell lysis, HA-**IKK** immunoprecipitation and determination of **IKK** activity. Data are expressed as the fold increase in

kinase activity relative to the basal activity and are means.+-.range of single determinations pooled from three experiments. See FIG. 3. Control immune-**complex** kinase assays performed with a mutated GST-I.kappa.B(1-62) (serines 32 and 36 mutate to alanines) failed to support phosphorylation. Hence, like IKK.alpha., IKK.alpha..DELTA.C is also a cytokine stimulated I.kappa.B kinase.

Detailed Description Text - DETX (132):

IKK.alpha. and IKK.alpha..DELTA.C possess functional domains (see FIG. 1). These functional domains are known to play a role in protein-protein association. Klemm et al., Annu. Rev. Immunol. 16, 569-592 (1988). Indeed, IKK.alpha. was identified as being a component of a high molecular weight cytoplasmic **complex** by several groups. Chen et al., Cell 84, 853-862 (1996); DiDonato et al., Nature 388, 548-554 (1997); Lee et al., Cell 88, 213-222 (1997). Co-transfection assays demonstrate that IKK.alpha. and IKK.beta. co-immunoprecipitate with themselves and each other, see Mercurio et al., Science 278, 860-866 (1997); Woronicz et al. (1997); and Zandi et al. (1997), suggesting that either the protein **complex** precipitates with each **IKK** or that IKK.alpha. and IKK.beta. form both homo- and hetero-dimers. Since IKK.alpha..DELTA.C was lacking the **IKK** H-L-H domain, we performed sucrose-density gradient (SDG) centrifugation analysis of IKK.alpha., IKK.alpha..DELTA.C, and IKK.beta. to determine if each kinase behaved in a similar fashion.

Detailed Description Text - DETX (134):

Under the experimental conditions employed, more than 90% of the IKK.alpha. was observed to migrate in SDG as a dimer or greater, with 10% of the proteins migrating as a monomeric species. See FIG. 4. Migration of SDG-resolved IKK.alpha. (CHUK) showed the protein to bind to additional proteins present in the reticulocyte lysate. This was unlikely to be due to non-specific aggregation as the presence of 4 M Urea in SDS-PAGE gels failed to modify the IKK.alpha. migration. Hence, IKK.alpha. most probably exists as a dimer or a larger oligomer. In contrast, IKK.alpha..DELTA.C was seen to migrate in SDG as a monomeric species (>90% of the detectable protein). Furthermore, IKK.alpha..DELTA.C failed to associate to a significant extent with either IKK.alpha. or IKK.beta. as the addition of either IKK.alpha. or IKK.beta. did not appreciably alter the migration profile of IKK.alpha..DELTA.C in SDG. Experiments were performed at both 40.degree. C. and 25.degree. C. as this may affect dimerisation, with comparable results being obtained. Additional experiments revealed that IKK.alpha. and IKK.beta. migrated at the same relative positions in SDG, when added singly or together, suggesting that they associate in the same **complex**. These results show that IKK.alpha..DELTA.C is a functional **IKK** but not via a direct interaction with either IKK.alpha. or IKK.beta..

Other Reference Publication - OREF (7):

Zandi et al., "The I.kappa.B Kinase **Complex (IKK**) Contains Two Kinase

Subunits, IKK.alpha. and IKK.beta., Necessary for I.kappa.B Phosphorylation and NF-.kappa.B Activation", Cell vol. 91: 243-252 (1997).

US-PAT-NO: 6066474

DOCUMENT-IDENTIFIER: US 6066474 A

TITLE: Y2H56 A strong IKK binding protein

DATE-ISSUED: May 23, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Marcu; Kenneth B.	Stony Brook	NY	N/A	N/A

APPL-NO: 09/ 195286

DATE FILED: November 18, 1998

US-CL-CURRENT: 435/69.1, 435/252.3 , 435/320.1 , 530/350 , 536/23.1 , 536/23.5

ABSTRACT:

The present invention provides an isolated I.kappa.B kinase binding protein designated Y2H56 and functional equivalents therof. The amino acid sequence of Y2H56, the nucleotide sequence encoding Y2H56, and other related protein and nucleic acid molecules are also provided.

8 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Other Reference Publication - OREF (8):

Zandi et al., "The I.kappa.B Kinase **Complex (IKK**) Contains Two Kinase Subunits, IKK.alpha. and IKK.beta., Necessary for I.kappa.B Phosphorylation and NF-.kappa.B Activation", Cell 91:243-252 (1997).

US-PAT-NO: 5977341

DOCUMENT-IDENTIFIER: US 5977341 A

TITLE: Antisense modulation of inhibitor-kappa B kinase-beta expression

DATE-ISSUED: November 2, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Monia; Brett P.	La Costa	CA	N/A	N/A
Cowsert; Lex M.	Carlsbad	CA	N/A	N/A

APPL-NO: 09/ 197008

DATE FILED: November 20, 1998

US-CL-CURRENT: 536/24.5, 435/375 , 435/440 , 435/6 , 435/91.1 , 536/23.1 , 536/24.3 , 536/24.33

ABSTRACT:

Antisense compounds, compositions and methods are provided for modulating the expression of Inhibitor-kappa B Kinase-beta. The compositions comprise antisense compounds, particularly antisense oligonucleotides, targeted to nucleic acids encoding Inhibitor-kappa B Kinase-beta. Methods of using these compounds for modulation of Inhibitor-kappa B Kinase-beta expression and for treatment of diseases associated with expression of Inhibitor-kappa B Kinase-beta are provided.

19 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Other Reference Publication - OREF (5):

Zandi et al., The IkappaB kinase **complex (IKK)** contains two kinase subunits, IKKalpha and IKKbeta, necessary for IkappaB phosphorylation and NF-kappaB activation, Cell, 1997, 91:243-252.

US-PAT-NO: 5972655

DOCUMENT-IDENTIFIER: US 5972655 A
See image for Certificate of Correction

TITLE: Y2H61 an IKK binding protein

DATE-ISSUED: October 26, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Marcu; Kenneth B.	Stony Brook	NY	N/A	N/A

APPL-NO: 09/ 196048

DATE FILED: November 19, 1998

US-CL-CURRENT: 435/69.1, 435/252.3 , 435/320.1 , 435/325

ABSTRACT:

The present invention provides an isolated I.kappa.B kinase binding protein designated Y2H61 and functional equivalents therof. The amino acid sequence of Y2H61, the nucleotide sequence encoding Y2H61, and other related protein and nucleic acid molecules are also provided.

5 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Other Reference Publication - OREF (9):

Zandi et al., "The I.kappa.B Kinase **Complex (IKK**) Contains Two Kinase Subunits, IKK.alpha. and IKK.beta., Necessary for I.kappa.B Phosphorylation and NF-.kappa.B Activation", Cell 91:243-252 (1997).

US-PAT-NO: 5962673

DOCUMENT-IDENTIFIER: US 5962673 A

TITLE: Antisense modulation of inhibitor-kappa B kinase-alpha expression

DATE-ISSUED: October 5, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Monia; Brett P.	La Costa	CA	N/A	N/A
Cowsert; Lex M.	Carlsbad	CA	N/A	N/A

APPL-NO: 09/ 197360

DATE FILED: November 20, 1998

US-CL-CURRENT: 536/24.5, 435/375 , 435/6 , 536/23.1 , 536/24.1 , 536/24.3

ABSTRACT:

Antisense compounds, compositions and methods are provided for modulating the expression of Inhibitor-kappa B Kinase-alpha. The compositions comprise antisense compounds, particularly antisense oligonucleotides, targeted to nucleic acids encoding Inhibitor-kappa B Kinase-alpha. Methods of using these compounds for modulation of Inhibitor-kappa B Kinase-alpha expression and for treatment of diseases associated with expression of Inhibitor-kappa B Kinase-alpha are provided.

20 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Other Reference Publication - OREF (5):

Zandi et al., The IkappaB kinase **complex (IKK)** contains two kinase subunits, IKKalpha and IKKbeta, necessary for IkappaB phosphorylation and NF-kappaB activation, Cell, 1997, 91:243-252.

US-PAT-NO: 5939302

DOCUMENT-IDENTIFIER: US 5939302 A

TITLE: IKK-.beta. proteins, nucleic acids and methods

DATE-ISSUED: August 17, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Goeddel; David V.	Hillsborough	CA	N/A	N/A
Woronicz; John	South San Francisco	CA	N/A	N/A

APPL-NO: 09/ 099124

DATE FILED: June 17, 1998

PARENT-CASE:

INTRODUCTION

This is a divisional application of U.S. Ser. No. 08/890,853, filed Jul. 10, 1997, which is a continuing application under 35USC120 of U.S. Ser. No. 08/887,114 filed Jul. 1, 1997, abandoned, both of which are incorporated herein by reference.

US-CL-CURRENT: 435/194

ABSTRACT:

The invention provides methods and compositions relating to an I.kappa.B kinase, IKK-.beta., and related nucleic acids. The polypeptides may be produced recombinantly from transformed host cells from the disclosed IKK-.beta. encoding nucleic acids or purified from human cells. The invention provides isolated IKK-.beta. hybridization probes and primers capable of specifically hybridizing with the disclosed IKK-.beta. genes, IKK-.beta.-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

6 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (7):

Here, we disclose a novel kinase I.kappa.B Kinase, **IKK**-.beta., as a NIK-interacting protein. **IKK**-.beta. has sequence similarity to the conceptual translate of a previously identified open reading frame postulated to encode a serine-threonine kinase of unknown function (`Conserved Helix-loop-helix Ubiquitous Kinase` or CHUK, Connelly and Marcu, 1995; Mock et al., 1995). Catalytically inactive mutants of **IKK**-.beta. are shown to suppress NF-.kappa.B activation induced by TNF and IL-1 stimulation as well as by TRAF and NIK overexpression; transiently expressed **IKK**-.beta. is shown to associate with the endogenous I.kappa.B.alpha. **complex; and IKK**-.beta. is shown to phosphorylate I.kappa.B.alpha. on serines 32 and 36. As used herein, Ser32 and Ser36 of I.kappa.B refers collectively to the two serine residues which are part of the consensus sequence DSGL/IXSM/L (e.g. ser 32 and 36 in I.kappa.B.alpha., ser 19 and 23 in I.kappa.B.beta., and ser 157 and 161, or 18 and 22, depending on the usage of methionines, in I.kappa.B.epsilon., respectively.

US-PAT-NO: 5916760

DOCUMENT-IDENTIFIER: US 5916760 A

TITLE: IKK-.beta. proteins, nucleic acids and methods

DATE-ISSUED: June 29, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Goeddel; David V.	Hillsborough	CA	N/A	N/A
Woronicz; John	South San Francisco	CA	N/A	N/A

APPL-NO: 09/ 099125

DATE FILED: June 17, 1998

PARENT-CASE:

This is a divisional application of U.S. Ser. No. 08/890,853, filed Jul. 10, 1997, now U.S. Pat. No. 5,851,812, which is a continuing application under 35USC120 of U.S. Ser. No. 08/887,114 filed Jul. 1, 1997, abandoned, both of which are incorporated herein by reference.

US-CL-CURRENT: 435/15, 435/194

ABSTRACT:

The invention provides methods and compositions relating to an I.kappa.B kinase, IKK-.beta., and related nucleic acids. The polypeptides may be produced recombinantly from transformed host cells from the disclosed IKK-.beta. encoding nucleic acids or purified from human cells. The invention provides isolated IKK-.beta. hybridization probes and primers capable of specifically hybridizing with the disclosed IKK-.beta. genes, IKK-.beta.-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

16 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (7):

Here, we disclose a novel kinase I.kappa.B Kinase, **IKK**-.beta., as a NIK-interacting protein. **IKK**-.beta. has sequence similarity to the conceptual

translate of a previously identified open reading frame postulated to encode a serine-threonine kinase of unknown function (`Conserved Helix-loop-helix Ubiquitous Kinase` or CHUK, Connelly and Marcu, 1995; Mock et al., 1995). Catalytically inactive mutants of **IKK**-.beta. are shown to suppress NF-.kappa.B activation induced by TNF and IL-1 stimulation as well as by TRAF and NIK overexpression; transiently expressed **IKK**-.beta. is shown to associate with the endogenous I.kappa.B.alpha. **complex; and IKK**-.beta. is shown to phosphorylate I.kappa.B.alpha. on serines 32 and 36. As used herein, Ser32 and Ser36 of I.kappa.B refers collectively to the two serine residues which are part of the consensus sequence DSGL/IXSMIL (e.g. ser 32 and 36 in I.kappa.B.alpha., ser 19 and 23 in I-.kappa.B.beta., and ser 157 and 161, or 18 and 22, depending on the usage of methionines, in I.kappa.B.epsilon., respectively.

US-PAT-NO: 5891719

DOCUMENT-IDENTIFIER: US 5891719 A

TITLE: IKAP nucleic acids

DATE-ISSUED: April 6, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cohen; Lucie	South San Francisco	CA	N/A	N/A
Baeuerle; Patrick	South San Francisco	CA	N/A	N/A

APPL-NO: 08/ 971244

DATE FILED: November 16, 1997

US-CL-CURRENT: 435/325, 435/252.3 , 435/254.11 , 435/69.1 , 530/300 , 530/350 , 536/23.1 , 536/23.5 , 536/24.3 , 536/24.31

ABSTRACT:

The invention provides methods and compositions relating to IKAP proteins which regulate cellular signal transduction and transcriptional activation, and related nucleic acids. The polypeptides may be produced recombinantly from transformed host cells from the disclosed IKAP encoding nucleic acids or purified from human cells. The invention provides isolated IKAP hybridization probes and primers capable of specifically hybridizing with the disclosed IKAP genes, IKAP-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

46 Claims, 1 Drawing figures

Exemplary Claim Number: 24

Number of Drawing Sheets: 1

---------- KWIC ---------

Brief Summary Text - BSTX (6):

The NF-.kappa.B-inducing kinase (NIK) is a member of the MAP kinase kinase kinase (MAP3K) family that was identified as a TRAF2-interacting protein (Malinin et al., 1997). NIK activates NF-.kappa.B when overexpressed, and kinase-inactive mutants of NIK comprising its TRAF2-interacting C-terminal domain (NIK.sub.(624-947)) or lacking two crucial lysine residues in its kinase domain (NIK.sub.(KK429-430AA)) behave as dominant-negative inhibitors that

suppress TNF-, IL-1-, and TRAF2-induced NF-.kappa.B activation (Malinin et al., 1997). Recently, NIK was found to associate with additional members of the TRAF family, including TRAF5 and TRAF6. Catalytically inactive mutants of NIK also inhibited TRAF5- and TRAF6-induced NF-.kappa.B activation, thus providing a unifying concept for NIK as a common mediator in the NF-.kappa.B signaling cascades triggered by TNF and IL-1 downstream of TRAFs. Recently two NIK-interacting protein designated characterized as novel human kinase I.kappa.B Kinases, **IKK**-.alpha. and **IKK**-.beta. have been reported (Woronicz et al., 1997; Mercurio et al. 1997; Maniatis, 1997). Catalytically inactive mutants of **IKK** suppress NF-.kappa.B activation induced by TNF and IL-1 stimulation as well as by TRAF and NIK overexpression; transiently expressed **IKK** associates with endogenous I.kappa.B.alpha. **complex; and IKK** phosphorylates I.kappa.B.alpha. on serines 32 and 36.

US-PAT-NO: 5854003

DOCUMENT-IDENTIFIER: US 5854003 A

TITLE: Screening method for agents that modulate human NIK activity

DATE-ISSUED: December 29, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rothe; Mike	San Mateo	CA	N/A	N/A
Wu; Lin	South San Francisco	CA	N/A	N/A

APPL-NO: / 032475

DATE FILED: February 26, 1998

PARENT-CASE:

This is a divisional application of U.S. Ser. No. 08/887,518, filed Jul. 3, 1997.

US-CL-CURRENT: 435/7.8

ABSTRACT:

The invention provides methods and compositions relating to a novel kinase, NIK, involved in NF.kappa.B activation. The polypeptides may be produced recombinantly from transformed host cells from the disclosed NIK encoding nucleic acids or purified from human cells. The invention provides isolated NIK hybridization probes and primers capable of specifically hybridizing with the disclosed NIK genes, NIK-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

9 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (7):

Here, we disclose a novel human NIK (NIK.sub.(Ala25)), which also provides the foregoing functionalities yet deviates in terms of critical sequence and structural characteristics; in particular, a Pro-Ala substitution at position 25 imposes altered protein structure. We show that the NIK.sub.(Ala25) variant

interacts with and cross-phosphorylates the I.kappa.B Kinases .alpha. and .beta., **IKK**-.alpha. and **IKK**-.beta. (see Goeddel et al. and Rothe et al., copending applications T97-006 and T97-007, respectively, filed Jul. 1, 1997). **IKK**-.alpha. and **IKK**-.beta. have sequence similarity to the conceptual translate of a previously identified open reading frame postulated to encode a serine-threonine kinase of unknown function (`Conserved Helix-loop-helix Ubiquitous Kinase` or CHUK, Connelly and Marcu, 1995; Mock et al., 1995). Catalytically inactive mutants of the **IKKs** suppress NF-.kappa.B activation induced by TNF and IL-1 stimulation as well as by TRAF and NIK overexpression; transiently expressed **IKKs** associate with the endogenous I.kappa.B.alpha. **complex; and the IKKs** phosphorylate I.kappa.B.alpha. on serines 32 and 36. As used herein, Ser32 and Ser36 of I.kappa.B refers collectively to the two serine residues which are part of the consensus sequence DSGL/IXSM/L (e.g. ser 32 and 36 in I.kappa.B.alpha., ser 19 and 23 in I.kappa.B.beta., and ser 157 and 161, or 18 and 22, depending on the usage of methionines, in I.kappa.B.epsilon., respectively. In addition, we disclose that NIK.sub.(Ala25) associates with other members of the TRAF family, including TRAF5 and TRAF6. Catalytically inactive mutants of NIK.sub.(Ala25) also inhibit TRAF5- and TRAF6-induced NF-.kappa.B activation, thus providing a unifying concept for NIK.sub.(Ala25) as a common mediator in the NF-.kappa.B signaling cascades triggered by TNF and IL-1 downstream of TRAFs.

US-PAT-NO: 5851812

DOCUMENT-IDENTIFIER: US 5851812 A

TITLE: IKK-.beta. proteins, nucleic acids and methods

DATE-ISSUED: December 22, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Goeddel; David V.	Hillsborough	CA	N/A	N/A
Woronicz; John	South San Francisco	CA	N/A	N/A

APPL-NO: 08/ 890853

DATE FILED: July 10, 1997

PARENT-CASE:

This a continuing application under 35USC120 of U.S. Ser. No. 08/887,114 filed Jul. 1, 1997, abandoned.

US-CL-CURRENT: 435/194, 435/252.3 , 435/325 , 536/23.2

ABSTRACT:

The invention provides methods and compositions relating to an I.kappa.B kinase, IKK-.beta., and related nucleic acids. The polypeptides may be produced recombinantly from transformed host cells from the disclosed IKK-.beta. encoding nucleic acids or purified from human cells. The invention provides isolated IKK-.beta. hybridization probes and primers capable of specifically hybridizing with the disclosed IKK-.beta. genes, IKK-.beta.-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

12 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (7):

Here, we disclose a novel kinase I.kappa.B Kinase, **IKK**-.beta., as a NIK-interacting protein. **IKK**-.beta. has sequence similarity to the conceptual translate of a previously identified open reading frame postulated to encode a serine-threonine kinase of unknown function (`Conserved Helix-loop-helix

Ubiquitous Kinase` or CHUK, Connelly and Marcu, 1995; Mock et al., 1995). Catalytically inactive mutants of **IKK**-.beta. are shown to suppress NF-.kappa.B activation induced by TNF and IL-1 stimulation as well as by TRAF and NIK overexpression; transiently expressed **IKK**-.beta. is shown to associate with the endogenous I.kappa.B.alpha. **complex; and IKK**-.beta. is shown to phosphorylate I.kappa.B.alpha. on serines 32 and 36. As used herein, Ser32 and Ser36 of I.kappa.B refers collectively to the two serine residues which are part of the consensus sequence DSGL/IXSM/L (e.g. ser 32 and 36 in I.kappa.B.alpha., ser 19 and 23 in I.kappa.B.beta., and ser 157 and 161, or 18 and 22, depending on the usage of methionines, in I.kappa.B.epsilon., respectively.

US-PAT-NO: 5844073

DOCUMENT-IDENTIFIER: US 5844073 A

TITLE: Human NIK proteins

DATE-ISSUED: December 1, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rothe; Mike	San Mateo	CA	N/A	N/A
Wu; Lin	South San Francisco	CA	N/A	N/A

APPL-NO: / 023321

DATE FILED: February 13, 1998

PARENT-CASE:

This is a divisional application of U.S. Ser. No. 08/887,518, filed Jul. 3, 1997.

US-CL-CURRENT: 530/300, 530/324 , 530/325 , 530/326 , 530/327 , 530/328 , 530/350

ABSTRACT:

The invention provides methods and compositions relating to a novel kinase, NIK, involved in NF.kappa.B activation. The polypeptides may be produced recombinantly from transformed host cells from the disclosed NIK encoding nucleic acids or purified from human cells. The invention provides isolated NIK hybridization probes and primers capable of specifically hybridizing with the disclosed NIK genes, NIK-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

6 Claims, 0 Drawing figures

Exemplary Claim Number: 2

---------- KWIC ---------

Brief Summary Text - BSTX (8):

Here, we disclose a novel human NIK (NIK .sub.(Ala25)), which also provides the foregoing functionalities yet deviates in terms of critical sequence and structural characteristics; in particular, a Pro-Ala substitution at position 25 imposes altered protein structure. We show that the NIK .sub.(Ala25)

variant interacts with and cross-phosphorylates the I.kappa.B Kinases .alpha. and .beta., **IKK**-.alpha. and **IKK**-.beta. (see Goeddel et al. and Rothe et al., copending applications T97-006 (U.S. Ser. No. 08/887,114) and T97-007 (U.S. Ser. No. 08/887,115, abandoned), respectively, filed Jul. 1, 1997). **IKK**-.alpha. and **IKK**-.beta. have sequence similarity to the conceptual translate of a previously identified open reading frame postulated to encode a serine-threonine kinase of unknown function (`Conserved Helix-loop-helix Ubiquitous Kinase` or CHUK, Connelly and Marcu, 1995; Mock et al., 1995). Catalytically inactive mutants of the **IKKs** suppress NF-.kappa.B activation induced by TNF and IL-1 stimulation as well as by TRAF and NIK overexpression; transiently expressed **IKKs** associate with the endogenous I.kappa.B.alpha. **complex; and the IKKs** phosphorylate I.kappa.B.alpha. on serines 32 and 36. As used herein, Ser32 and Ser36 of I.kappa.B refers collectively to the two serine residues which are part of the consensus sequence DSGL/IXSM/L (e.g. ser 32 and 36 in I.kappa.B.alpha., ser 19 and 23 in I.kappa.B.beta., and ser 157 and 161, or 18 and 22, depending on the usage of methionines, in I.kappa.B.epsilon., respectively. In addition, we disclose that NIK .sub.(Ala25) associates with other members of the TRAF family, including TRAF5 and TRAF6. Catalytically inactive mutants of NIK .sub.(Ala25) also inhibit TRAF5- and TRAF6-induced NF-.kappa.B activation, thus providing a unifying concept for NIK .sub.(Ala25) as a common mediator in the NF-.kappa.B signaling cascades triggered by TNF and IL-1 downstream of TRAFs.

US-PAT-NO: 5843721

DOCUMENT-IDENTIFIER: US 5843721 A

TITLE: Nucleic acids encoding human NIK protein

DATE-ISSUED: December 1, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rothe; Mike	San Mateo	CA	N/A	N/A
Wu; Lin	South San Francisco	CA	N/A	N/A

APPL-NO: 08/ 887518

DATE FILED: July 3, 1997

US-CL-CURRENT: 435/69.2, 435/243 , 435/320.1 , 435/325 , 435/410 , 435/69.1 , 536/23.1 , 536/23.5 , 536/24.31

ABSTRACT:

The invention provides methods and compositions relating to a novel kinase, NIK, involved in NF.kappa.B activation. The polypeptides may be produced recombinantly from transformed host cells from the disclosed NIK encoding nucleic acids or purified from human cells. The invention provides isolated NIK hybridization probes and primers capable of specifically hybridizing with the disclosed NIK genes, NIK-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.

15 Claims, 0 Drawing figures

Exemplary Claim Number: 3

---------- KWIC ---------

Brief Summary Text - BSTX (7):

Here, we disclose a novel human NIK(NIK .sub.(Ala25), which also provides the foregoing functionalities yet deviates in terms of critical sequence and structural characteristics; in particular, a Pro-Ala substitution at position 25 imposes altered protein structure. We show that the NIK.sub.(Ala25) variant interacts with and cross-phosphorylates the I.kappa.B Kinases .alpha. and .beta., **IKK**-.alpha. and **IKK**-.beta. (see Goeddel et al. and Rothe et al., copending applications T97-006 and T97-007, respectively, filed Jul. 1, 1997). **IKK**-.alpha. and **IKK**-.beta. have sequence similarity to the conceptual translate of a previously identified open reading frame postulated to encode a

serine-threonine kinase of unknown function (`Conserved Helix-loop-helix Ubiquitous Kinase` or CHUK, Connelly and Marcu, 1995; Mock et al., 1995). Catalytically inactive mutants of the **IKKs** suppress NF-.kappa.B activation induced by TNF and IL-1 stimulation as well as by TRAF and NIK overexpression; transiently expressed **IKKs** associate with the endogenous I.kappa.B.alpha. **complex; and the IKKs** phosphorylate I.kappa.B.alpha. on serines 32 and 36. As used herein, Ser32 and Ser36 of I.kappa.B refers collectively to the two serine residues which are part of the consensus sequence DSGL/IXSM/L (e.g. ser 32 and 36 in I.kappa.B.alpha., ser 19 and 23 in I.kappa.B.beta., and ser 157 and 161, or 18 and 22, depending on the usage of methionines, in I.kappa.B.epsilon., respectively. In addition, we disclose that NIK.sub.(Ala25) associates with other members of the TRAF family, including TRAF5 and TRAF6. Catalytically inactive mutants of NIK.sub.(Ala25) also inhibit TRAF5- and TRAF6-induced NF-.kappa.B activation, thus providing a unifying concept for NIK.sub.(Ala25) as a common mediator in the NF-.kappa.B signaling cascades triggered by TNF and IL-1 downstream of TRAFs.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	323	ikk$2	USPAT; US-PGPUB	2003/05/09 09:04
2	L2	538092	complex	USPAT; US-PGPUB	2003/05/09 09:04
3	L3	83	1 same 2	USPAT; US-PGPUB	2003/05/09 09:05
4	L4	17	spa-1	USPAT; US-PGPUB	2003/05/09 14:59
5	L5	2	1 and 4	USPAT; US-PGPUB	2003/05/09 14:59

PGPUB-DOCUMENT-NUMBER: 20030064408

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030064408 A1

TITLE: Protein-protein interactions

PUBLICATION-DATE: April 3, 2003

US-CL-CURRENT: 435/7.1, 435/194 , 435/7.92 , 530/388.26

APPL-NO: 10/ 035343

DATE FILED: January 4, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60259572 20010104 US

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is related to U.S. provisional patent application Serial No. 60/259,572, filed on Jan. 4, 2001, incorporated herein by reference, and claims priority thereto under 35 USC .sctn.119(e).

PGPUB-DOCUMENT-NUMBER: 20030036095

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030036095 A1

TITLE: Highly sensitive proteomic analysis methods, and kits and systems for practicing the same

PUBLICATION-DATE: February 20, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Tchaga, Grigoriy S.	Newark	CA	US	

APPL-NO: 09/ 960716

DATE FILED: September 21, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60234527 20000922 US

US-CL-CURRENT: 435/7.1, 427/2.11 , 435/7.9

ABSTRACT:

Methods of determining whether a sample includes one or more analytes, particularly proteinaceous analytes, of interest are provided. In the subject methods, an array of binding agents, where each binding agent includes an epitope binding domain of an antibody, is contacted with the sample. In many embodiments, contact occurs in the presence of a metal ion chelating polysaccharide, e.g., a pectin. Following contact, the presence of binding complexes on the array surface are detected and the resultant data is employed to determine whether the sample includes the one or more analytes of interest. Also provided are kits, systems and other compositions of matter for practicing the subject methods. The subject methods and compositions find use in a variety of applications, including proteomic applications such as protein expression analysis, e.g., differential protein expression profiling.

[0001] CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] Pursuant to 35 U.S.C. .sctn.119(e), this application claims priority to the filing date of U.S. Provisional Patent Application Serial No. 60/234,527 filed Sep. 22, 2000, the disclosure of which is herein incorporated by reference.

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Detail Description Table CWU - DETL (1):

1 Antibody Table 14-3-3 e v.2 53BP2 v.2 ABP-280 ABR ACH ESTERASE ACH rec B ACTR/AIB1/RAC3 adaptin alpha adaptin beta ADAPTIN d v.3 Adaptin gamma AF6/p180 AFAP AIM-1 AKAP 149 AKAP 79 AKAP-KL ALDH alpha-/beta-SNAP AMPHIPHYSIN AMPK b v.2 Annexin II Annexin XI AP 180 v.2 ApoE APOLIPOPROTEIN App-BP1 ARF-3 B100 ARGINASE I ATAXIN-2 v.2 ATTRACTIN B CATENIN B NAP v.2 B56-alpha BAG-1/RAP46 BCL-2 Bcl-xl beta 1 Ca channel beta-Arrestin 1 BM28 v.2 BMXv.2 Bog bPIX BRAMP2/AMPHIPHYSIN 2 BRM BRUCE Btf c-cbl C-NAP1 Cadherin (5) Cadherin (E) Cadherin (P) CAF1 p150 CALCINEURIN Calnexin CALRETININ v.2 Calsequestrin CAM KIN KIN v.2 Casein K1e casein kin.IIb Casein kinase 1 Catenin (alpha) Cathepsin D Cathepsin L epsilon Caveolin 2 CDC 34 CDC 37 CDC27 hs CDC42GAP (Rac1) CDK 2 CDK4 CDK7 cGB-PDE v.2 CHD3 CHGB Chromogranin A/CGA CIP1 CLA-1 Clathrin HC CLIP-115 COL7A1 COMT Contactin v.2 COX-2 CPG16/DCAMKL1 CRIK CRP-1 Csk v.3 CUL-2 v.2 CUL-3 CYCLIN D3 Cypher1 DAP3 DARPP-32 v.2 DBP2 DBP2 DDX-1 DEK DEMATIN v.2 DFF45 DGKTHETA v.2 DHFR DLG-1 v.2 DLP1 DMPK DNA Polymerase DNA Polymerase e Doublecortin v.2 delta (catalytic) DSIF DYNAMIN 1 v.2 Dynamin II DYRK EB1 EBP5O v.2 EEA-1 eEF-2 kinase Efp Eg5 EGF Recept EGF Recept (activated) EIF-4 gamma eIF-4E eIF-5A Endoglin Endothelin 1 Recept. EphA4/ELF-1 EPS-8 ERG2 ERp72 v.2 ESE1 v.3 Exportin-1/CRM1 EXPORTIN-t v.3 Ezrin FADD v.2 Fas Fas Ligand FBP v.2 FEN-1 FIN13 FKBP 12 FKBP51 FKBP65 FLAP v.2 Flotilin-2/ESA FNK v.2 Frabin FXR2 v.2 FYB v.2 G3BP GABAbR2 v.2 GAGE GAP 1 m Gelsolin Gephyrin GLUCOCORTICOID GM-CSF GOK GPI-phoslipase D. GRIP GS-15 v.2 GSK 3D GSPT2 GST pl Guanylate Kinase HAP1 v.2 HAX-1 hcKROX HDAC3 HDJ-2 HEME OXYGENASE HHR23B HIC-5 v.2 1 HIF-1a HIF1b/ARNT1 hILP (H59520) hILP (H62120) hPRP16 v.2 hPRP17 HRAD9 HS-1 Hsp-90 Hsp10 Hsp110 HSP70 v.2 IFN-.Yen. Human IFN-.Yen. Rat IKKb IKKg/NEMO IL-10 IL-12p40 IL-12 p70 IL-13 IL-1.beta. IL-2 IL-2 sRd IL-3 IL-4 IL-5 IL-6 IL-8 INHIBITOR 2 iNOS/MAC NOS Integrin .ae butted.5 IP3 rec IQGAP IRS-1 v.6 ISGF3 p48 ITCH JNKK1/MKK4 K CHANNEL a SUB. Kalinin B1 Karyopherin B KATANIN p80 Ki-67 KIF3B v.2 KRIP-1 KSR-1 v.3 Ku70 L-Caldesmon LA PROTEIN LAIR-1 v.2 Lamp1/CD107a LAR-PTP LAT v.2 LCK LFA-1 alpha LSP1 LXRa v.3 M33 MAP4 MCC MCM5 MCP-1 MDC9 Mek5 Melusin MENA MINT1 v.2 MINT3/XII gamma MITOSIN MKK7 v.2 MONA MRE II MSH3 MSH6/GTBP MST-1 MST-3 MUPP1 v.2 MXI-1 MYR6 NABC1/AIBC1 NASP v.2 NEDD-4 Nek 2 Nek3 NES-1 NESTIN Neurexin I NEUROGENIN 3 NEUROGLYCAN v.2 NEUROPILIN-2 NEXILIN NF kappa beta v.2 NHE-1 NHE-3 Nhe-3 v.3 NIP1 NM23 NMDAR2B v.2 nNOS/NOS Type1 NTF2 NUCLEPORIN P62 v.2 NuMA Nup88 p116 RIP v.2 p19 SKP1 p190-B v.3 p36 p38 delta v.2 p47 PHOX p52/LEDGF P53 p54nrb p56 dok2 P62DOK PARP Paxillin PCMT-II PCNA PDGF Rec PDI PECI PER2 Pericentrin Pex1 v.2 PEX19 PhLP PI3-KINASE p110a PI4-Kinase Beta PIN v.6 PIP5Kg PKC EPSILON PKC iota PKC LAMBDA* PKC THETA PKR v.3 PLAKOPHILIN 2a PLC beta 1 v.2 PLC delta 1 PLK PMF-1 PNUTS PRK2 v.2 R cadherin v.2 RAB 27 RAB-5 RAB11 RAB4 Rabphilin 3A RACK 1 RAP2 RAS (Ha) RAS-GRF2 v.2 RB2 RCH-1 REF-1 RNAse H1 ROAZ v.2 ROK alpha sCD23 SCP3 SH2-B v.2 Shc C SII SIII p15 SIP1 SKAPP55 v.2 SLP76 SMAD2 v.2 SMAD4 SMN v.2 SNX1 v.3 SNX2 SPA-1 v.2 SPOT 14 SQS SRP54 SRPK1 SSeckS STAT-3 STAT6/IL-4 STAT v.5 STI1 SYNAPTOGYRIN Synaptophysin Synaptotagmin Syntaxin 4 SYNTAXIN 6 v.2 TAF 70-alpha TAF-172 Tat-SF1 TENSIN TFII-1 TGF-.beta. THROMBOSPONDIN 1 TIAR TIEG2 TLS TNF-.oe butted. TOPO IIa v.2 TOPO IIb TPL-2 TRADD v.2 TRAX TREX 1 TRF2 TRP1 UBE3A V-1/myotrophin VASP Veli1 VESL-1L VHR VLA-3 alpha VTI1b SUBUNIT WRN XIN XPD ZAP-70K ZBP-89 ZFP-37 ZO-1

L Number	Hits	Search Text	DB	Time stamp
-	323	ikk$2	USPAT; US-PGPUB	2003/05/09 09:04
-	538092	complex	USPAT; US-PGPUB	2003/05/09 09:04
-	83	ikk$2 same complex	USPAT; US-PGPUB	2003/05/09 15:01
-	17	spa-1	USPAT; US-PGPUB	2003/05/09 14:59
-	2	ikk$2 and spa-1	USPAT; US-PGPUB	2003/05/09 14:59
-	4	spa-1 same complex	USPAT; US-PGPUB	2003/05/09 15:01

PGPUB-DOCUMENT-NUMBER: 20030064477

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030064477 A1

TITLE: Novel E6 targeted protein (E6TP1)

PUBLICATION-DATE: April 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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APPL-NO: 10/ 215050

DATE FILED: August 8, 2002

RELATED-US-APPL-DATA:

child 10215050 A1 20020808

parent continuation-of 09362336 19990728 US GRANTED

parent-patent 6440696 US

US-CL-CURRENT: 435/69.2, 435/184 , 435/320.1 , 435/325 , 536/23.2

ABSTRACT:

The invention relates to a putative human tumor suppressor protein identified as a novel GAP protein, designated "E6TP1" (for E6-targeted protein), its nucleic acid and amino acid sequences, and methods of use thereof in the regulation of small G protein signaling pathways. In addition, methods of use of E6TP1 as a Therapeutic for treatment or prevention of carcinomas, especially HPV-associated carcinomas of anogenital origin, and other diseases is encompassed in the invention.

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Detail Description Paragraph - DETX (194):

[0220] Homology analysis of E6TP1 polypeptide sequence. Although the E6TP1 sequence represent a novel cDNA sequence, a Gapped BLASTP search of the NCBI database showed that E6TP1 residues 489-819 share high sequence identity with GAP domains of known and putative RapGTPase-activating proteins (GAPs). The proteins with highest homology to E6TP1 include the mammalian GAPs Rap1GAP, tuberin (the tuberous sclerosis **complex** 2 product, TSC2), and **SPA-1** (FIGS. 2C

and D), as well as Drosophila Rapgap1 and two putative RapGAPs in C. elegans, predicted by the open reading frames identified in genomic sequences (Table 1). FIG. 2C represents an amino acid sequence alignment of E6TP1.alpha. to known GAP proteins (e.g., human tuberin, human **SPA-1,** and human Rap1GAP), wherein homology comparisons were made using the Clustral algorithm with a gap penalty of 3 and refined by manual adjustment. The schematic in FIG. 2D illustrates our alignment of E6TP1 with human **SPA-1**. Numbers indicate the beginning and ending amino acid positions of homologous regions. Percent homology is indicated with each region. Homology with **SPA-1** extends beyond the GAP domain and includes the putative leucine zipper region (47% identity between E6TP1.alpha. aa 1705-1779 or E6TP1.beta. aa 1726-1790 versus **SPA-1** residues 963 to 1027), as well as other regions, with an overall 42% amino acid identity between E6TP1 residues 319-1205 and **SPA-1** residues 104-930. See, e.g., Kurachi et al., J Biol Chem-272: 28081-28088 (1997).

PGPUB-DOCUMENT-NUMBER: 20030064408

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030064408 A1

TITLE: Protein-protein interactions

PUBLICATION-DATE: April 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Heichman, Karen	Salt Lake City	UT	US	
Bartel, Paul L.	Salt Lake City	UT	US	

APPL-NO: 10/ 035343

DATE FILED: January 4, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60259572 20010104 US

US-CL-CURRENT: 435/7.1, 435/194 , 435/7.92 , 530/388.26

ABSTRACT:

The present invention relates to the discovery of novel protein-protein interactions that are involved in mammalian physiological pathways, including physiological disorders or diseases. Examples of physiological disorders and diseases include non-insulin dependent diabetes mellitus (NIDDM), neurodegenerative disorders, such as Alzheimer's Disease (AD), and the like. Thus, the present invention is directed to complexes of these proteins and/or their fragments, antibodies to the complexes, diagnosis of physiological generative disorders (including diagnosis of a predisposition to and diagnosis of the existence of the disorder), drug screening for agents which modulate the interaction of proteins described herein, and identification of additional proteins in the pathway common to the proteins described herein.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is related to U.S. provisional patent application Serial No. 60/259,572, filed on Jan. 4, 2001, incorporated herein by reference, and claims priority thereto under 35 USC .sctn.119(e).

---------- KWIC ---------

Claims Text - CLTX (2):

1. An isolated protein **complex** comprising two proteins, the protein **complex** selected from the group consisting of: (i) a **complex** of a first protein and a second protein; (ii) a **complex** of a fragment of said first protein and said second protein; (iii) a **complex** of said first protein and a fragment of said second protein; and (iv) a **complex** of a fragment of said first protein and a fragment of said second protein, wherein said first and second proteins are selected from the group consisting of: (a) said first protein is IKKb and said second protein is selected from the group consisting of LDHM, EIF3S10, SLAP2, KIAA0614, SART-1 and GBDR1; (b) said first protein is IKKa and said second protein is GBDR1; (c) said first protein is IKKg and said second protein is TRAF; and (d) said first protein is IKK-i and said second protein is selected from the group consisting of NUMA1, **SPA-1** and PN13730.

PGPUB-DOCUMENT-NUMBER: 20030044783

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030044783 A1

TITLE: Human genes and gene expression products

PUBLICATION-DATE: March 6, 2003

INVENTOR-INFORMATION:

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Sudduth-Klinger, Julie	Alameda	CA	US	
Reinhard, Christoph	Oakland	CA	US	
Randazzo, Filippo	San Francisco	CA	US	
Kennedy, Giulia C.	Arlington	VA	US	
Pot, David	Oakland	CA	US	
Kassam, Altaf	Moraga	CA	US	
Lamson, George	Palo Alto	CA	US	
Drmanac, Radjoe	Hollister	CA	US	
Dickson, Mark	Mountain View	CA	US	
Labat, Ivan	Sunnyvale	CA	US	
Jones, Lee William	Sunnyvale	CA	US	
Stache-Crain, Birgit			US	

APPL-NO: 09/ 803719

DATE FILED: March 9, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60188609 20000309 US

US-CL-CURRENT: 435/6, 435/183 , 435/320.1 , 435/325 , 435/69.1 , 530/350 , 530/388.1 , 536/23.2

ABSTRACT:

This invention relates to novel human polynucleotides and variants thereof, their encoded polypeptides and variants thereof, to genes corresponding to these polynucleotides and to proteins expressed by the genes. The invention also relates to diagnostic and therapeutic agents employing such novel human polynucleotides, their corresponding genes or gene products, e.g., these genes and proteins, including probes, antisense constructs, and antibodies.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. provisional application serial No. 60/188,609, filed Mar. 9, 2000, which application is incorporated herein by reference in its entirety.

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Detail Description Table CWU - DETL (43):

509 114441 ATP SYNTHASE A CHAIN (PROTEIN 6) 3.6.1.34) protein 6 - fruit 3 fly (Drosophila yakuba) mitochondrion (SGC4) >gi.vertline.12921.vertline.emb.vertline- .CAA25442.vertline.(X00924) ATPase subunit 6 [Drosophila 510 1280094 (U55369) No definition line found [Caenorhabditis elegans] 3 511 226131 thyroid hormone receptor alpha 2 [Rattus norvegicus] 3 512 3860855 (AJ235271) GUANOSINE PENTAPHOSPHATE 2.9 PHOSPHOHYDROLASE (gppA) [Rickettsia prowazekii] 513 584834 CELLULOSE SYNTHASE OPERON C PROTEIN xylinus] 2.4 >gi.vertline.1090660.vertline.prf.vertline..vertline.2019362C acsC gene 514 629777 chitinase (EC 3.2.1.14) - barley vulgare] 2.2 515 2555183 (AF026504) **SPA-1** like protein p1294 [Rattus norvegicus] 2.2 516 4503737 forkhead (Drosophila) homolog 1 HEAD DOMAIN PROTEIN 1.8 FKHR >gi.vertline.631145.vertline.pir.vertline..vertline.- S40521 FKHR protein - human >gi.vertline.435423 (U02310) fork head domain protein [Homo sapiens] >gi.vertline.737918.vertli- ne.prf.vertline..vertline.1923399A FKHR gene [Homo sapiens] 517 3790719 (AF099916) contains similarity to C2H2-type zinc fingers 1.8 518 1785942 (U83412) CAG [Drosophila melanogaster] 1.8 519 3877036 (Z81079) predicted using Genefinder; similar to collagen; cDNA 1.4 EST EMBL:M88890 comes from this gene; cDNA EST EMBL:Z14325 comes from this gene; cDNA EST EMBL:D27520 comes from this gene; cDNA EST EMBL:D72240 comes from this 520 281654 hypothetical protein 24 - Agrobacterium tumefaciens plasmid 1.3 pTi15955 >gi.vertline.39086.vertline.emb.vertline.CAA25186.vertline.tu- mefaciens] 521 114972 BETA-GLUCOSIDASE (GENTIOBIASE) Ruminococcus albus 1.3 >gi.vertline.45968.vertline.emb.vertline.CAA33461.v- ertline.(X15415) beta-glucosidase (AA 1-947) [Ruminococcus albus] albus] 522 1707085 (U80451) Similar to collagen [Caenorhabditis elegans] 1.3 523 483163 nonstructural protein - hepatitis E virus RNA-directed RNA 1.3 polymerase [Hepatitis E virus] 524 4455275 (AL035527) putative protein [Arabidopsis thaliana] 0.99 525 4376875 (AE001642) CT465 hypothetical protein 0.45 526 2494911 HYPOTHETICAL PROTEIN KIAA0124 product is novel. [Homo 0.44 527 4539280 (AL049498) putative transcription factor 0.28 528 1842255 (U74613) hepatocyte nuclear factor-3/fork head homolog 11B [Homo 0.28 529 231977 D(4) DOPAMINE RECEPTOR (D(2C) DOPAMINE RECEPTOR) 0.073 >gi.vertline.203916 (M84009) dopamine receptor D4 530 987050 (X65335) lacZ [Cloning vector pSV-beta-Galactosidase Control] 0.042 531 2493416 S100 CALCIUM-BINDING PROTEIN A13 calcium-binding protein 0.031 A13 (S100A13) [Homo sapiens] 532 1778844 (U83086) LimA [Dictyostelium discoideum] 0.0006 533 3288470 (AJ224360) surf5c [Homo sapiens] 5e-015 534 3947614 (AL023828) cDNA EST yk491f8.5 comes from this gene 1e-015 [Caenorhabditis elegans] 535 1086860 (U41272) Similar to man(9)-alpha-mannosidase. 3e-028 536 3875451 (Z66496) cDNA EST EMBL:D71941 comes from this gene; cDNA 2e-030 EST EMBL:D74691 comes from this gene; cDNA EST EMBL:D76330 comes from this gene; cDNA EST

EMBL:D65192 comes from this gene; cDNA EST EMBL:D68540 comes from this 537
3877493 (Z48583) similar to ATPases associated with various cellular 1e-035
activities (AAA); cDNA EST EMBL:Z14623 comes from this gene; cDNA EST
EMBL:D75090 comes from this gene; cDNA EST EMBL:D72255 comes from this gene;
cDNA EST yk200e4.5... 538 3169010 (AJ006412) putative GTP-binding protein
2e-042 539 3877493 (Z48583) similar to ATPases associated with various
cellular 4e-044 activities (AAA); cDNA EST EMBL:Z14623 comes from this gene;
cDNA EST EMBL:D75090 comes from this gene; cDNA EST EMBL:D72255 comes from
this gene; cDNA EST yk200e4.5... 540 3877493 (Z48583) similar to ATPases
associated with various cellular 3e-044 activities (AAA); cDNA EST EMBL:Z14623
comes from this gene; cDNA EST EMBL:D75090 comes from this gene; cDNA EST
EMBL:D72255 comes from this gene; cDNA EST yk200e4.5... 608 3882189
(AB018277) KIAA0734 protein [Homo sapiens] 9.9 609 3877937 (Z48716)
similarity to a transmembranous region of ubiquinol- 9.6 cytochrome-C
reductase (PIR accession number S38960); cDNA EST EMBL:T00461 comes from this
gene; cDNA EST EMBL:D27071 comes from this gene; cDNA EST EMBL:D27070 610
3643019 (AF064703) glucose transporter 1; CeGT1 [Drosophila 8.4 611 3219946
HYPOTHETICAL PROTEIN MJ1394 Methanococcus jannaschii 8 >gi.vertline.1592041
(U67579) conserved hypothetical protein 612 3219946 HYPOTHETICAL PROTEIN
MJ1394 Methanococcus jannaschii 8 >gi.vertline.1592041 (U67579) conserved
hypothetical protein 613 2833328 FIBRILLARIN 7.9 614 4505481 nucleoporin 88
kD **complex** protein [Homo sapiens] 7.8 615 220578 (D00570) open reading frame
(251 AA) [Mus musculus] 7.8 616 266810 NAD(P) TRANSHYDROGENASE SUBUNIT BETA
7.6 transhydrogenase [Escherichia coli] transhydrogenase (B-specific) (EC
1.6.1.1) b chain NAD(P)+transhydrogenase (B-specific) (EC (1.6.1.1) b chain
[Escherichia coli] >gi.vertline.1787886 (AE000255) pyridine 617 807646
(M17294) unknown protein [Human herpesvirus 4] 7.6 618 829186 (X03879)
rudimentary protein fragment 7.4 619 4322346 (AF081825) sodium-dependent
high-affinity dicarboxylate 7.4 transporter [Rattus norvegicus] 620 3334785
(AL031107) hypothetical protein SC5A7.04c 7.4 621 1346720
PHOSPHATIDYLINOSITOL-4-PHOSPHATE 5-KINASE TYPE II 7.3 ALPHA (PIP5KII-ALPHA)
KINASE) >gi.vertline.1079474.vertline.pir.vertline..ve- rtline.A55967 1-
phosphatidylinositol-4-phosphate 5-kinase (EC 2.7.1.68) - human
>gi.vertline.758697 (U14957) 53 K isoform of Type II
phosphatidylinositol-4- 622 4105819 (AF050175) Rab7 [Homo sapiens] 6.4 623
155865 (M93125) 80 kDa protein [Babesia bovis] 6.3 624 2133638 boule protein
- fruit fly (Drosophila melanogaster) >gi.vertline.1395211 6.2 (U51858)
boule protein 625 1788052 (AE000270) putative transport system permease
protein 6.2 626 3875616 (Z77657) F08H9.9 [Caenorhabditis elegans] 6.2 627
2499150 HYPOTHETICAL PROTEIN IN CPS REGION 6.2 628 1170758 GALECTIN-3
(GALACTOSE-SPECIFIC LECTIN 3) (MAC-2 6.1 ANTIGEN) (IGE-BINDING PROTEIN) (35
KD LECTIN) (CARBOHYDRATE BINDING PROTEIN 35) (CBP 35) 629 2495335 HEAT
SHOCK
PROTEIN 42 (42 KD HEAT SHOCK PROTEIN) 6
>gi.vertline.1077219.vertline.pir.vertline..- vertline.S49767 heat shock
protein HSP42 - yeast (Saccharomyces cerevisiae) 630 1086677 (U41020) coded
for by C. elegans cDNA yk64f5.3; coded for by C. 6 elegans cDNA yk64f5.5;
Similar to zinc finger. [Caenorhabditis 631 1170758 GALECTIN-3
(GALACTOSE-SPECIFIC LECTIN 3) (MAC-2 6 ANTIGEN) (IGE-BINDING PROTEIN) (35 KD
LECTIN) (CARBOHYDRATE BINDING PROTEIN 35) (CBP 35) 632 440957 Achaete-Scute
homolog Mash-1 gene product 6 633 1786037 (U72284) NADH dehydrogenase subunit
2 [Apis mellifera] 6

US-PAT-NO: 6440696

DOCUMENT-IDENTIFIER: US 6440696 B1

TITLE: E6 targeted protein (E6TP1)

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INVENTOR-INFORMATION:

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ABSTRACT:

The invention relates to a putative human tumor suppressor protein identified as a novel GAP protein, designated "E6TP1" (for E6-targeted protein), its nucleic acid and amino acid sequences, and methods of use thereof in the regulation of small G protein signaling pathways. In addition, methods of use of E6TP1 as a Therapeutic for treatment or prevention of carcinomas, especially HPV-associated carcinomas of anogenital origin, and other diseases is encompassed in the invention.

19 Claims, 17 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

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Detailed Description Text - DETX (196):

Although the E6TP1 sequence represent a novel cDNA sequence, a Gapped BLASTP search of the NCBI database showed that E6TP1 residues 489-819 share high sequence identity with GAP domains of known and putative RapGTPase-activating proteins (GAPs). The proteins with highest homology to E6TP1 include the mammalian GAPs Rap1GAP, tuberin (the tuberous sclerosis **complex** 2 product, TSC2), and **SPA-1** (FIGS. 2C and D), as well as Drosophila Rapgap1 and two putative RapGAPs in C. elegans, predicted by the open reading frames identified in genomic sequences (Table 1). FIG. 2C represents an amino acid sequence alignment of E6TP1.alpha. to known GAP proteins (e.g, human tuberin, human

SPA-1, and human Rap1GAP), wherein homology comparisons were made using the Clustral algorithm with a gap penalty of 3 and refined by manual adjustment. The schematic in FIG. 2D illustrates our alignment of E6TP1 with human **SPA-1**. Numbers indicate the beginning and ending amino acid positions of homologous regions. Percent homology is indicated with each region. Homology with **SPA-1** extends beyond the GAP domain and includes the putative leucine zipper region (47% identity between E6TP1.alpha. aa 1705-1779 or E6TP1.beta. aa 1726-1790 versus **SPA-1** residues 963 to 1027), as well as other regions, with an overall 42% amino acid identity between E6TP1 residues 319-1205 and **SPA-1** residues 104-930. See, e.g., Kurachi et al., J Biol Chem 272: 28081-28088 (1997).